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Chemical and Physical Characteristics of Phosphate Rock Materials of Varying Reactivity

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Several chemical and physical properties of 10 phosphate rock (PR) materials of varying reactivity were evaluated. These included North Carolina phosphate rock (NCPR), Sechura phosphate rock (SPR), Chatham Rise phosphorite (CRP), Arad phosphate rock (APR), Gafsa phosphate rock (GPR), North Florida phosphate rock (NFPR), Jordanian phosphate rock (JPR), Mexican phosphate rock (MPR), Nauru Island phosphate rock (NIPR), and Makatea Island phosphate rock (MIPR). Concentrations of As and Cd were highest in NCPR and U was higher in APR and CRP than in the other reactive PR materials. Because Cd and U can accumulate in biological systems, it may be necessary to direct more attention towards the likely implications of Cd and U concentrations when evaluating a PR for direct application. Three sequential extractions with 2% citric acid may be more useful for comparing the chemical solubility of PR materials, particularly for those containing appreciable $CaCO_3$. For each particle-size fraction from <63 to >500 μm, SPR was more soluble in 2% citric acid than was NCPR. The higher solubility of SPR than NCPR in 2% citric acid, 2% formic acid, and neutral ammonium citrate possibly results from a higher chemical reactivity rather than from any difference in the surface area presented for dissolution. The poor relationship obtained between surface area and the solubility of the PR materials in the three chemical extractants suggests that surface area plays a secondary role to chemical reactivity in controlling the solubility of a PR in a chemical extractant. A Promesh plot provided an effective method for describing the particle-size characteristics of those PR materials which occurred as sands. Fundamental characteristics, such as mean particle size and uniformity, can readily be determined from a Promesh plot.

Keywords: Solubility in extractants; sequential extraction; Promesh plot; particle size; surface area; trace elements; phosphate rock.

1. Introduction

Phosphate rock (PR) materials vary appreciably in their 'chemical reactivity', a term which is widely and often rather loosely used to describe the solubility of a PR in certain chemical extractants and its potential agronomic effectiveness. Variations in chemical reactivity have been attributed primarily to differences in the degree of substitution of carbonate for phosphate (P) in the apatite structure.[1,2] Measurement of the degree of carbonate substitution, the concentration of major elements, and solubility in certain extractants form the basis on which the agronomic evaluation of a PR for direct application is generally based.[3,4] The concentration of elements such as arsenic (As), cadmium (Cd), and uranium (U), all of which can be found in high concentrations in PR materials,[5] and all of which can be toxic to man,[6,7] is not generally included in the chemical analysis. Because Cd and U can accumulate in biological systems[6,7] continued exposure to even low concentrations of these elements

may ultimately present a higher health hazard than infrequent exposure to high concentrations from sources such as mining, industrial activity, or sewage disposal. There is a need for information on the concentration of elements such as As, Cd, and U, particularly in those PR materials which show potential as direct application phosphate fertilisers.

The chemical reactivity of PR materials has often been assessed by their solubility in chemical extractants.[4,8] However, the solubility of a PR which contains significant amounts of calcite cannot accurately be assessed by a single chemical extraction procedure.[8] The neutralising effect of free calcium carbonate results in an underestimate of extractable P and when solubility in a particular reagent forms the basis for establishing chemical reactivity[8] the true chemical reactivity of the PR is underestimated. Increasing the strength of the extracting solution[4,8] or the solution:solid ratio[9] only appears to overcome the effect of the presence of small amounts of calcite. The solubility of a PR is also affected by the surface area of the dissolving particle.[1,10] The extent to which differences in surface area, over and above differences in chemical reactivity, can modify the dissolution of a PR does not appear to have been investigated to any significant extent.

Several reactive PR materials, such as North Carolina phosphate rock (NCPR) and Sechura phosphate rock (SPR) occur as sands. In addition to a saving in grinding costs, these unground materials may offer advantages in handling and spreading, relative to finely-ground materials. Characterising these materials to ensure physical quality and to prevent excessive fines or tailings in the material requires more than one particle size measurement. This immediately leads to difficulties in interpreting the results of particle-size analyses. Promesh plots,[11] a plot of particle size (log scale) against cumulative per cent undersize (probability scale), may offer a rapid and effective method for describing the particle-size characteristics of unground PR materials.

The objectives of this study were to compare several chemical and physical properties of 10 PR materials, which include the major sources of reactive PR in the world, and to evaluate the use of a Promesh plot as a method for describing the particle-size characteristics of PR materials which occur as fine sands.

2. Experimental

The 10 PR materials used and their origin are listed in Table 1. Three of the PR materials, NCPR, SPR, and Mexican phosphate rock (MPR), were received unground. The remaining PR materials had been ground.

2.1. Chemical analysis

For all chemical analyses a composite sample of each PR was ground to 100%<250 μm. All chemical analyses were done in duplicate. Following digestion in HCl/HNO_3, P was determined colorimetrically; Ca, Mg, K, Na, Fe, Al, Mn, Co, Cu, Zn, and Cd were determined by atomic absorption spectrophotometry; and S gravimetrically as $BaSO_4$. Calcium carbonate was determined manometrically following CO_2 evolution after acid digestion. Fluorine, extracted by fusion with

Table 1. Name and origin of phosphate rock materials

Phosphate rock	Abbreviation	Origin
Gafsa	GPR[a]	Tunisia
North Florida	NFPR[a]	North Florida, USA
Jordanian	JPR[a]	Jordan
North Carolina	NCPR[b]	North Carolina, USA
Sechura	SPR[b]	Sechura Desert, Peru
Mexican	MPR[b]	Mexico
Nauru Island	NIPR[a]	Nauru Island, South Pacific
Arad	APR[a]	Negev Desert, Israel
Makatea Island	MIPR[a]	Makatea Island, South Pacific
Chatham Rise phosphorite	CRP[a]	Chatham Rise, New Zealand

Received as [a]ground and [b]unground.

NaOH pellets, was liberated with 0.4 M sodium citrate and measured by a specific fluoride electrode. Following digestion in $H_2SO_4/HClO_4$, As was liberated from the digest with sodium borohydride and determined by atomic absorption spectrophotometry. Uranium was determined by delayed neutron activation analysis using the Australian Atomic Energy Commission research reactor, MOATA. Uranium-235 is the only naturally occurring isotope which can be fissioned by thermal neutrons. Such fission events can be detected specifically by counting the delayed neutron emission from the fission products.

The solubility of the 10 PR materials in 2% formic acid,[12] 2% citric acid,[13] and neutral ammonium citrate (NAC)[14] was measured on a <250-μm sample. After the first extraction with citric acid, the residue was extracted a second and a third time with fresh citric acid. The solubility in 2% citric acid was determined for unground SPR and NCPR, plus seven particle-size fractions (<63, 63–125, 125–150, 150–180, 180–250, 250–500, and >500 μm) of each of these two materials.

2.2. Surface area determinations and morphology

Surface area was determined on a 125–150 μm particle size fraction and on an 'as received' sample of each PR. Each PR was heated to 100°C in a vacuum before carrying out the surface area determination. Heating to 100°C did not result in any structural changes, based on X-ray powder diffraction. The surface area determinations were carried out with N_2 gas at the temperature of liquid N_2 using the Brunauer, Emmett, and Teller (BET) method.[15]

2.3. Particle-size determination

Particle-size characteristics of the three unground PR materials (NCPR, MPR, and SPR) were determined by the end-point method,[16] using the rate test. Each PR was placed on top of a nest of four sieves (75, 125, 250, and 500 μm) with a lid and receiver, and shaken by machine for 5 min. The 500 μm sieve was removed, inverted over a sheet of paper, and cleaned. The rate test was carried out by returning the residue to the clean 500 μm sieve, shaking, and tapping by hand for 2 min. If the amount of PR passing the sieve was <0.2% by weight of the sample, sieving was regarded as complete. If >0.2% of the PR passed the sieve, the sample was shaken for another 5 min, and the rate test repeated. The PR passing this sieve was placed on the remaining nest of sieves and shaken for another 5 min. The rate test was then conducted on the 250-μm sieve. The remaining sieves were treated in the same way. In a Promesh plot, particle size (log scale) is plotted against cumulative per cent undersize (probability scale). The Promesh plot was developed for use in the detergent industry by Falivene[10] to ease the interpretation of particle-size measurement. From such a plot, fundamental characteristics such as mean-particle size and uniformity can readily be calculated.

3. Results and discussion

3.1. Chemical composition

The amounts of several major and minor elements in the 10 PR materials are listed in Table 2. The concentrations of the major elements Ca, P, Na, Mg, and F in North Florida phosphate rock (NFPR), Jordanian phosphate rock (JPR), NCPR, SPR, Gafsa phosphate rock (GPR), and Nauru Island phosphate rock (NIPR) are in general agreement with previously published analyses.[17,18] Published data for the composition of APR[19] and, in particular, the MPR deposit are limited because these two have only been mined in recent years. Except for Chatham Rise phosphorite (CRP), which contains appreciable amounts of $CaCO_3$, the P content of the remaining PR materials varied from only 12.7 to 15.6% (Table 2). The CRP deposit is variable in both P (6 to 10%) and $CaCO_3$ (20 to 40%) content.[20,21]

The range in concentrations of Mn (2–212 mg kg^{-1}) is in agreement with previous reports,[22] as is the range in concentration of Co (4–6 mg kg^{-1}), Cu (4–15 mg kg^{-1}),[23] Zn (57–1010 mg kg^{-1}),[5] Cd (2–100 mg kg^{-1}),[24,25] As (1.5–22.6 mg kg^{-1}),[26] and U (68.9–153.3 mg kg^{-1}).[27] The concentration of As in NCPR (22.6 mg kg^{-1}) was four times higher than the average concentration (5.5 mg kg^{-1}) of the other nine PR materials (Table 2). The concentration of Cd in NIPR was 10 times higher than in SPR and CRP. Nauru Island phosphate rock has for many years been used in Australia and New

Table 2. Chemical composition of phosphate rock materials

Phosphate rock	Element (g kg^{-1})										Element (mg kg^{-1})						
	Ca	P	Mg	K	Na	Fe	Al	S	F	CO_3[a]	Mn	Co	Cu	Zn	Cd	As	U
GPR	353	134	2.5	1.8	12	1.8	2.4	8	41	71	7	3	15	393	38	4	88
NFPR	333	133	2.4	0.9	6	9.2	4.7	5	40	40	212	5	4	57	3	7	143
JPR	365	134	1.4	0.3	6	2.2	2.8	6	38	77	5	<1	8	235	4	12	72
NCPR	341	127	3.1	0.8	9	4.6	2.1	9	35	117	7	2	9	400	48	23	69
SPR	334	131	3.2	0.1	16	3.2	3.6	16	34	51	91	3	6	178	11	5	72
MPR	357	140	1.1	1.2	4	2.8	4.7	3	41	60	151	6	4	90	8	3	118
NIPR	374	156	2.9	0.4	2	4.1	3.2	4	30	41	122	6	8	1010	100	3	64
APR	374	141	1.2	0.1	4	0.7	1.0	10	40	82	2	4	12	560	12	7	153
MIPR	374	130	2.6	0.2	8	0.7	0.6	2	32	162	2	<1	6	220	10	2	65
CRP	341	89	4.5	5.0	9	30.0	3.0	6	30	276	100	4	5	95	2	n.d.	101

n.d.=not determined.
[a]Expressed as $CaCO_3$.

Zealand for the manufacture of superphosphate. At present it constitutes about 40% of the PR used for superphosphate manufacture in these two countries. Elevated Cd levels were also found in NCPR and to a lesser extent GPR. Cadmium has been found to accumulate in the cultivated layer of agricultural soils[28] and in some instances increases in the Cd content of agricultural crops have been reported.[29] Although both NCPR and SPR are reactive PR materials,[4,8] the higher concentrations of both As and Cd in NCPR might reduce the attractiveness of this PR as a long-term, direct-application phosphate fertiliser.

Both NCPR and SPR contained similar amounts of U, but less than GPR and CRP, and only half that of APR. Six separate samples of SPR had U contents ranging from 70.3 to 73.6 mg kg^{-1} with a mean of 72.0, suggesting that the SPR deposit is relatively uniform in terms of U composition. Uranium has also been found to accumulate in topsoil after long-term superphosphate application.[30] Superphosphate, depending on the origin of the PR and the PR:acid ratio used in manufacture, can contain from 24 to 42 mg U kg^{-1}.[30] Use of CRP or APR as a substitute for superphosphate, would result in a two- and three-fold increase, respectively, in the U accumulation rate. Although the long-term effect of continued exposure to low levels of U is not known, it is clear than U, along with As and Cd, should be given attention in future, when assessing the long-term suitability of a PR for direct application.

3.2. Chemical solubility

The 2% formic acid reagent, which has been found to be a better indicator of chemical reactivity than either 2% citric acid or NAC,[4,8] ranked SPR, NCPR, and GPR as reactive, APR and JPR as moderately reactive, and MPR, CRP, NFPR, NIPR, and MIPR as unreactive PR materials (Figure 1). Apart from CRP, which contains appreciable amounts of $CaCO_3$ (Table 2), the formic acid extractant separated the reactive PR materials from those which are unreactive and unsuitable for direct application.[4,8] Chatham Rise phosphorite, which has been found to be an effective P source in greenhouse[21,31] and field[32] investigations, was ranked as an unreactive PR by a single formic acid extraction. Three sequential extractions with 2% citric acid overcame the neutralising affect of the $CaCO_3$ in CRP. By ranking CRP as a reactive PR along with NCPR, SPR, and GPR (Figure 1), sequential extraction appears to be a useful method for comparing the chemical solubility of all PR materials, regardless of their $CaCO_3$ content. Although agronomic studies with APR are limited,[33] this PR is probably more reactive than JPR, but less so than either SPR or NCPR.

With all three extractants, and even after three sequential extractions with 2% citric acid, SPR was more soluble than NCPR, GPR, and CRP (Figure 1). In fact for each particle-size fraction from <63 to >500 μm, SPR was more soluble in 2% citric acid than NCPR (Figure 2). A comparison of the 125–150 μm particle size fraction of these two materials suggests that the difference in solubility results from the higher chemical reactivity of SPR, rather than from a difference in surface area (Table 3) which might be expected to influence dissolution. The solubility in 2% citric acid of

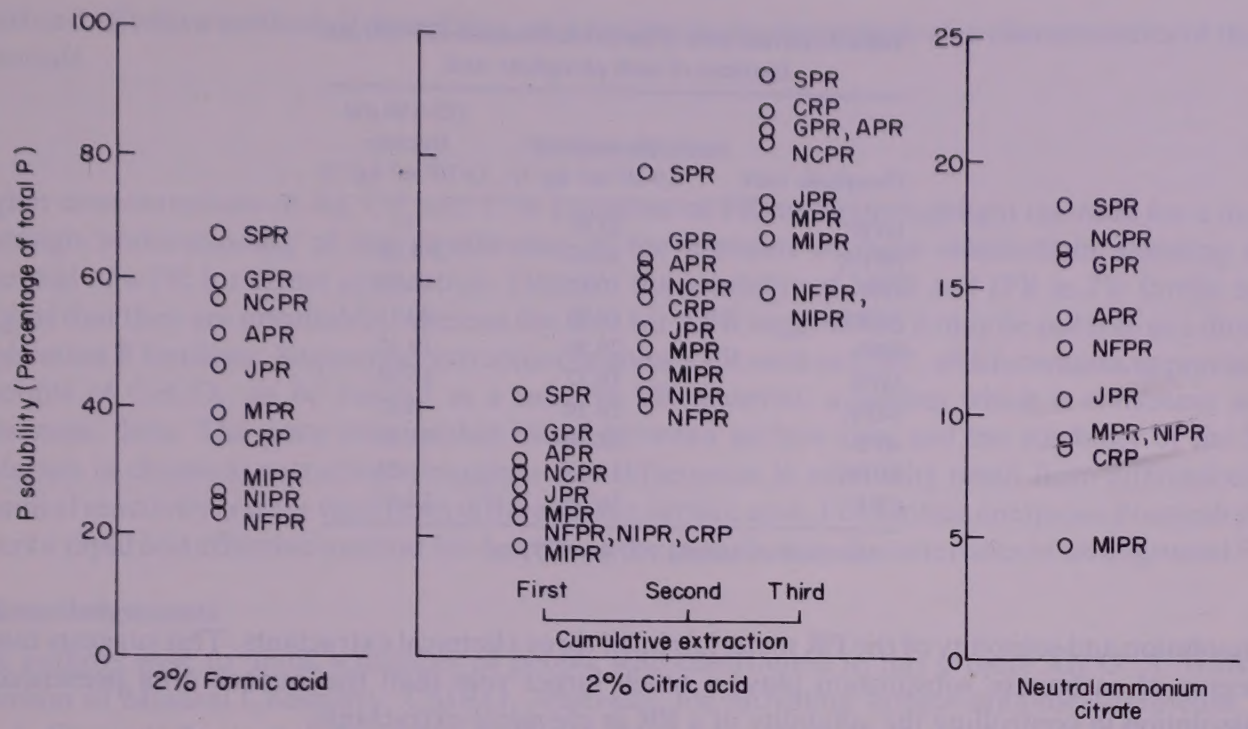

Figure 1. Solubility of phosphate rock materials in 2% formic acid, 2% citric acid, and NAC.

unground SPR was also higher than that of unground NCPR (Figure 2). The larger surface area of unground SPR indicates that this PR contains more finer particle than NCPR (Table 3). The lower surface area of the 125–150 μm particle-size fraction of NIPR and NFPR, and to a lesser extent SPR, indicates that the bulk of the as received sample was <125 μm, whereas a large part of as received CRP, MPR, NCPR, JPR, and APR was in the 125–150 μm particle-size range (Table 3). In contrast, MIPR had a much higher proportion of particles >150 μm (Table 3).

The surface area of the 125–150 μm fraction of APR was much higher than that of either NCPR or SPR. Although the surface are of APR was three times higher than that of JPR, and 1.6 and 1.7 times higher than that of NCPR and SPR, respectively, formic acid ranked this PR as being only moderately reactive (Figure 1). A poor relationship was found between the surface area for

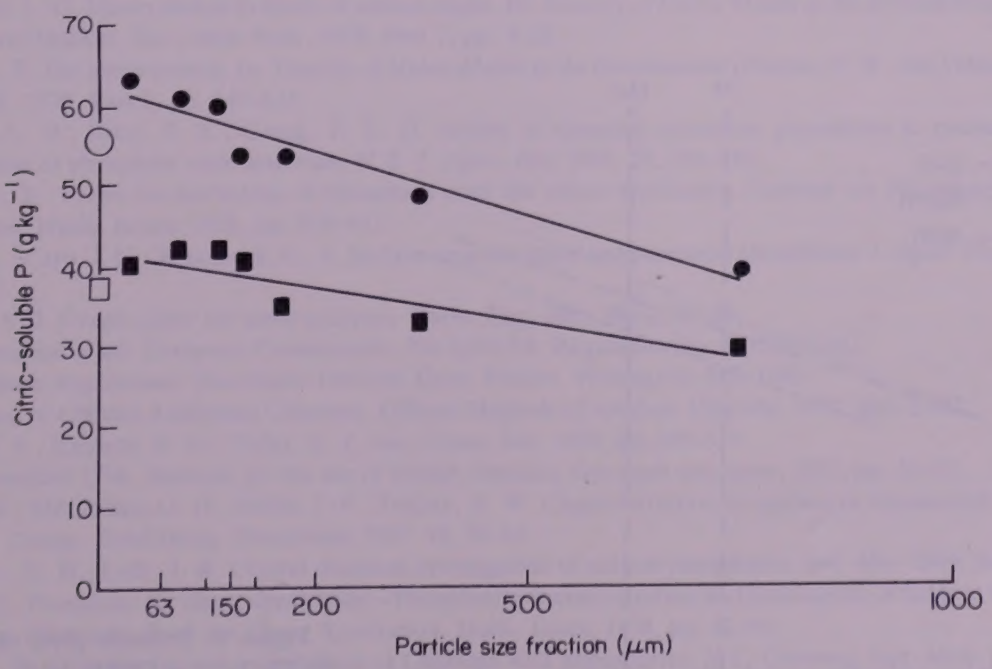

Figure 2. Amounts of citric-soluble P in unground SPR (○) and NCPR (□) and in seven particle-size fractions (<63, 63–125, 125–150, 150–180, 250–500, and >500 μm) of these two PR materials: (●), SPR; (■), NCPR.

Table 3. Surface area of the as received and 125–150 μm fractions of each phosphate rock

Phosphate rock	As received ($\times 10^3$ m^2 kg^{-1})	125–150 μm fraction ($\times 10^3$ m^2 kg^{-1})
GPR[a]	23.87	—
NFPR	16.82	4.08
JPR	10.85	12.13
NCPR	18.81	20.15
SPR	29.56	19.15
MPR	18.17	15.94
NIPR	18.10	6.67
APR	32.30	31.47
MIPR	18.17	15.94
CRP	16.94	13.65

[a]As received sample 100% <125 μm.

dissolution and solubility of the PR materials in all three chemical extractants. This suggests that the degree of carbonate substitution plays a much larger role than the surface area presented for dissolution in controlling the solubility of a PR in chemical extractants.

3.3. Particle-size characteristics

The straight line of a Promesh plot (Figure 3) has obvious advantages for assessing particle-size distribution over the more traditional cumulative percent oversize/undersize methods which plot as sigmoidal curves on semilog paper. If the particle-size fractions do not plot as a straight line, or the line is seriously distorted at either end, then the material is probably a blend or contains excessive fines or tailings. The geometric mean and uniformity (σ), defined as the ratio of the 84% probability value (Mσ) to the 50% value (M), of each PR can readily be determined from the Promesh plot.

The geometric mean of the three unground PR materials varied from 142 μm for SPR to 267 μm for NCPR. North Carolina phosphate rock (σ=1.33) was more uniform than either MPR (1.40) or SPR (σ=1.58). By establishing tolerances for the geometric mean and for uniformity, the Promesh plot could be used to set an envelope, within which a PR must remain for registration as a direct-application P fertiliser, should particle-size considerations be deemed important. If unground PR materials, such as SPR and NCPR, are found to be effective P sources, then the Promesh plot offers a

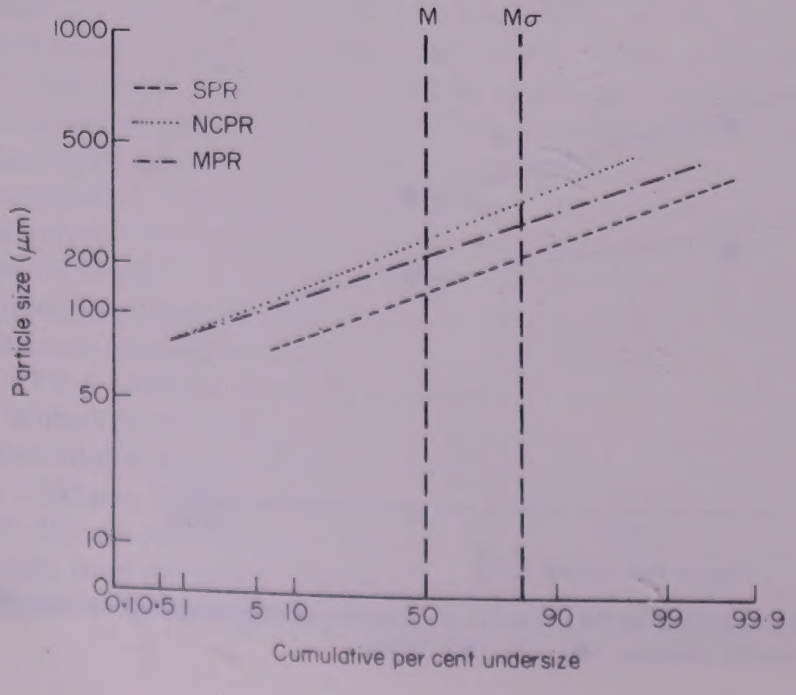

Figure 3. Promesh plots of unground (····) NCPR, (–·–·–) MPR, and (– – –), SPR.

rapid and effective method of describing, on a routine basis, the particle-size characteristics of these materials.

4. Conclusions

Higher concentrations of As, Cd, and U in a number of PR materials highlight the need for a more thorough understanding of the significance of the amounts of these elements in assessing the potential of a PR for direct application. Data for the solubility of MPR and JPR in 2% formic acid suggest that they are unsuitable, whereas the data for APR suggest that it may be suitable as a direct-application P fertiliser. Sequential extraction enables a PR such as CRP, which contains appreciable amounts of $CaCO_3$, to be ranked as a reactive PR material, a finding which is consistent with agronomic data. The poor relationship found between surface area and the solubility of the PR materials in chemical extractants, suggests that differences in solubililty result from differences in chemical reactivity, rather than from differences in surface area. For routine analyses a Promesh plot offers a rapid and effective method for describing the particle-size characteristics of an unground PR.

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Comparison of Methods to Describe the Kinetics of Digestion and Passage of Fibre in Sheep

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A mathematical model is described which was developed with the objective of analysing and interpreting data from rumen-emptying studies to estimate rates of digestion (k_d) and passage (k_p) in sheep offered hay once daily. It assumed that first-order kinetics applied to digestion in and passage from the rumen within a 24 h feeding cycle. The model predictions of k_d and k_p were compared with rates of digestion estimated by incubation of the hays in dacron bags and rates of passage determined using chromium-mordanted hay, in the same experiments. The predictions for rates of passage determined over the period 5–15 h postfeeding were lower than those determined from the chromium-mordant data and the simulations indicated that the fractional rate of passage during feeding was higher than that recorded in the postfeeding period. This suggests either that more than one fractional rate constant applies within a 24 h period, or that first-order kinetics do not apply. The estimates for k_d were considerably higher than those determined in dacron bags. Evaluation of the ability of the combined dacron bag and mordant techniques to estimate extent of digestion by comparison with in-vivo digestibility values indicated that the extent of digestion was underestimated. The predictions obtained from the rumen-emptying data were much closer to the in-vivo values.

Keywords: Fibre; model; rate of passage; rate of digestion; rumen emptying; dacron bag; chromium mordant.

1. Introduction

The degree of fill in the reticulo-rumen has been suggested as the dominant factor limiting voluntary intake of poorer quality forage diets, as these diets have relatively long rumen retention times.[1, 2] However, decreasing the retention time by increasing the rate of passage tends to decrease the extent of fibre digestion in the rumen[3] and hence in-vivo digestibility of the diet. Thus, there is a need to study the combined effects of digestion and passage to maximise the intake of digestible nutrients.

Waldo *et al.*[4] developed a mathematical model to predict the combined effects of digestion and passage on cellulose disappearance from the rumen and further models of this type have been reported by Mertens and colleagues.[5, 6] However, these models are based on rates of passage and digestion calculated from observations taken over a number of days and thus do not take into account the removal of fibre from the rumen within a day. The mechanisms which affect digestion and passage during and after a specific feeding period are likely to be important in controlling between-meal intervals[7] and may also influence daily intake.[8]

An alternative method for measuring the rate of removal of fibre from the rumen is to empty and sample the rumen contents at specific times after feeding. Separation of the fibre into digestible and indigestible fractions should then enable calculation of rate constants for both digestion and passage. In this paper, a model is described which was developed with the primary objective of analysing data obtained from such rumen-emptying studies to estimate rates of digestion and passage in sheep

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offered hay once daily. The collection of such data is time-consuming as rumen-emptying involves a major disruption to the rumen environment and at least two days should be allowed between emptyings. Hence, this approach was undertaken to examine the kinetics of fibre digestion in more detail, rather than to develop a routine technique. A second objective of the model was to use its predictions to test the assumptions on which more conventional techniques are based.

2. The model

2.1. Mathematical description

The model is illustrated in Figure 1 and applies to the behaviour of the neutral detergent fibre (NDF) fraction of the diet in the rumen, partitioned into its digestible (X_D) and indigestible (X_I) components. The indigestible fraction may be removed from the rumen only by passage; the digestible fraction may be removed by both digestion and passage. The sum of the removal of the two fractions is used as a measure of the total removal of fibre from the rumen. The dynamic properties of the system are defined by two linear first-order differential equations:

$$\frac{dX_I}{dt} = F_I - k_p X_I; \qquad 0 \leq t \leq t_f, \tag{1a}$$

$$= -k_p X_I; \qquad t_f < t \leq 24, \tag{1b}$$

where X_I=indigestible fraction of NDF (g); F_I=intake of indigestible fibre (g h^{-1}). F_I is assumed a constant, and feeding occurs once daily at a constant rate over the interval $0 \leq t \leq t_f$; k_p=fractional rate of passage of fibre from the rumen (h^{-1}); t_f=observed eating time.

Similarly,

$$\frac{dX_D}{dt} = F_D - (k_d + k_p) X_D; \qquad 0 \leq t \leq t_f, \tag{2a}$$

$$= -(k_d + k_p) X_D; \qquad t_f < t \leq 24, \tag{2b}$$

where X_D=digestible fraction of NDF (g); F_D=intake of digestible fibre (g h^{-1}), and is also constant, k_d=fractional rate of digestion of fibre in the rumen (h^{-1}).

The analytic solutions to equations (1) and (2) are as follows:

$$X_I = [X_I(0) - F_I/k_p] \exp(-k_p t) + F_I/k_p; \qquad 0 \leq t \leq t_f, \tag{3a}$$

$$= \{X_I(0) + [\exp(k_p t_f) - 1] F_I/k_p\} \exp(-k_p t); \qquad t_f < t \leq 24, \tag{3b}$$

$$X_D = [X_D(0) - F_D/(k_d + k_p)] \exp[-(k_d + k_p)t] + F_D/(k_d + k_p), \qquad 0 \leq t \leq t_f, \tag{4a}$$

$$= [X_D(0) - \{\exp[(k_d + k_p)t_f] - 1\} F_D/(k_d + k_p)] \exp[-(k_d + k_p)t]; \qquad t_f < t \leq 24. \tag{4b}$$

The $X(0)$s denote the initial values of the digestible and indigestible fibre fractions (i.e. the rumen pool sizes of the NDF fractions immediately prior to feeding). Since the Xs, $X(0)$s and Fs are

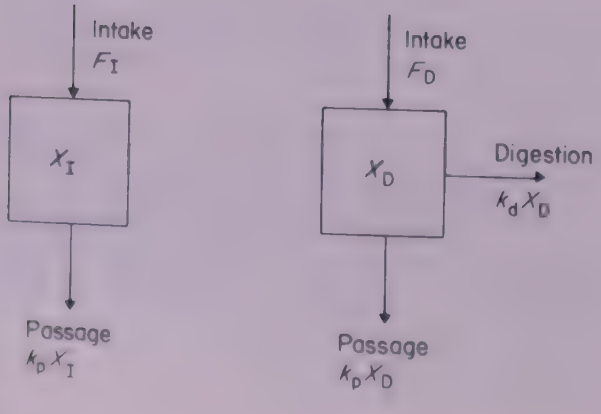

Figure 1. Model of the removal from the rumen of digestible (X_D) and indigestible (X_I) fibre.

experimentally determined, equations (3) and (4) can be fitted to the experimental data to determine the rate constants k_d and k_p. The values obtained may then be used to estimate the extent of digestion as follows.

Consider a quantity of fibre X ingested into the rumen at time $t=0$. Let the proportion of indigestible NDF present in X be denoted as f. Therefore:

$$X_I = fX, \tag{5}$$

$$X_D = (1-f)X. \tag{6}$$

For a unit quantity of X,

$$X_I(0)+X_D(0)=1. \tag{7}$$

The digestible fraction of this unit quantity of X will be subjected to both digestion and passage, therefore its kinetics will be as follows:

$$\frac{dX_D}{dt} = -(k_D+k_p)X_D, \qquad 0 \leqslant t < \infty. \tag{8}$$

Integration of equation (8) gives

$$X_D = (1-f)\exp[-(k_d+k_p)t]. \tag{9}$$

The extent of digestion of the fibre in the rumen, P, is therefore given by

$$P = \int_0^\infty k_d X_D \, dt = k_d(1-f)\int_0^\infty \exp[-(k_d+k_p)t]\, dt, \tag{10a}$$

$$= k_d(1-f)/(k_d+k_p). \tag{10b}$$

Thus, the model can be defined by three equations: equation (3b) which is used to determine rate of passage, equation (4b) which is used to determine rate of digestion, and equation (10b) which is used to estimate extent of digestion.

2.2. Assumptions used

As rumen digestion is a complex process, certain simplifying assumptions had to be made and these are discussed here in relation to both their validity and the restrictions they impose.

(a) The first assumption was that the processes of digestion and passage obey first-order kinetics and that the rate constants do not vary within a 24 h period. This implies that a constant proportion of the amount of substance present is digested or undergoes passage per unit time.

The kinetics of digestion were investigated by Gill *et al.*,[9] who observed linear semilog plots of cellulose digestion, which indicated first-order kinetic behaviour of the digestible cellulose fraction. Other work[10–13] has confirmed these observations, however, non-linear digestive behaviour during early fermentation has also been observed in some of these studies, indicating a delay before the onset of first-order behaviour.

Evidence that the rate of passage of digesta from the rumen obeys first-order kinetics arises from the interpretation of data on the faecal excretion of markers[14–16] The fractional rates of passage obtained in this way are mean values for the daily passage of marker from the rumen, and as such do not take account of any diurnal variation.

(b) The second assumption was that the cell wall fraction of the diet can be considered as two fractions, *viz.* a potentially digestible (X_d) and an indigestible fraction (X_I). This distinction is necessary as first-order kinetic digestion applies only to the digestible fraction of fibre; it was assumed that the indigestible fraction could only leave the rumen by passage. This distinction is a mathematical one and does not imply that the two pools are physically separate.

Evidence for the presence of a totally indigestible fraction of cell wall comes from the long-term in-vivo and in-vitro digestion studies, in which the disappearance of cell wall with time appears to

reach an asymptotic value after extended incubation (for example references 10 and 17). In this study the indigestible fraction was taken as the NDF remaining after a 96 h in-vitro incubation of the samples. This time was chosen to cover the major digestion period, although it is recognised that some further digestion may occur.[18]

(c) The third assumption is that the passage rates out of the rumen of both the digestible and the indigestible fractions are identical. While there is some evidence that large indigestible fibres (lignin) may be selectively retained,[3, 19] breakdown and digestion of feed within the rumen does not completely separate the two fractions into individual entities.[20] Thus, it seems reasonable to assume that the passage rates of the two fractions are very similar.[18]

2.3. Application

The model was applied to data on the fibre content of the rumen in sheep offered perennial ryegrass hays of different digestibility and a clover hay, with or without a maize starch supplement (Tables 1 and 2), as measured by emptying the rumen at specific times *after* once-daily feeding.[21, 22] Equation (3b) was fitted to the amounts of indigestible NDF (INDF) to calculate the rate of passage from the rumen (k_p). This value was then used in fitting equation (4b) to the amounts of digestible NDF (DNDF) to calculate the rate of digestion (k_d). These values for k_p and k_d, together with the proportion of indigestible fibre in the diet (f), were then used to estimate the extent of digestion in the rumen from equation (10b).

The limited number of sampling points did not always permit equation (3b) and (4b) to be fitted directly using non-linear least-squares, hence in some cases the fitting of equation (3b) and (4b) to the data was achieved by logarithmic transformation to the form:

$$\ln y = a - bt, \tag{11}$$

where b represents k_p in equation (3b) and (k_p+k_d) in equation (4b); a represents a constant term, and y is the appropriate fibre content at each emptying time t. This form (equation (11)) can be fitted to the data using the method of linear least-squares. The estimates thus obtained were termed k_p^* and k_d^* and the extent of digestion P^*.

3. Comparison of model predictions with those obtained by conventional methods

3.1. Rates of digestion and passage

Once estimates of the rate constants for digestion and passage had been obtained from the model, the second objective was to compare these values with estimates obtained by alternative methods to

Table 1. Experiment 1: rates of passage and digestion and extent of digestion in the rumen of the NDF fraction of the diet, obtained by application of the model to data from the experiment reported by Aitchison *et al.*,[21] also estimates of the parameters obtained from dacron bag and Cr-mordant studies, and comparisons between the two methods of estimation

	Treatment				Significance		Method comparison		
	EG	LG	WC	s.e.m.	EG *v* LG	Grasses *v* WC	Bias	s.e.m.[a]	Significance
Rates of passage (h^{-1})									
Model k_p^*	0.0108	0.0101	0.0181	0.00284	n.s.	n.s.			
Mordant k_p'	0.0332	0.0354	0.0298	0.00288	n.s.	n.s.	−0.0198	0.0028	***
Rates of digestion (h^{-1})									
Model k_d^*	0.147	0.096	0.181	0.0187	n.s.	*			
Dacron bag k_d'	0.0295	0.0268	0.0480	0.00284	n.s.	***	+0.1065	0.0134	***
Extent of digestion [g (g NDF intake)$^{-1}$]									
Model P^*	0.787	0.618	0.622	0.0235	***	*			
Dacron bag/mordant P'	0.469	0.383	0.543	0.0369	n.s.	*	+0.2110	0.0370	***
Estimated from in-vivo digestibilities	0.748	0.619	0.797	0.0120	***	***	—	—	—

[a] Standard error of mean bias calculated from differences between observations from two methods (17 d.f.).

n.s. Not significant

Treatments were perennial ryegrass hay cut early (EG) or late (LG) in the season, and white clover hay (WC).

Table 2. Experiment 2: rates of passage and digestion, and extent of digestion in the rumen, of the NDF fraction of the diet, obtained by application of the model to data from the experiment reported by Aitchison *et al.*,[22] also estimates of the parameters obtained from dacron bag and Cr-mordant studies, and comparisons between the two methods of estimation

	Treatment					Significance		Method comparison		
	H	HS	L	LS	s.e.m.	Level	Starch	Bias	s.e.m.[a]	Signifi-cance
Rates of passage (h^{-1})										
Model k_p^*	0.0071	0.0124	0.0064	0.0103	0.00392	n.s.	n.s.	−0.0269	0.00364	***
Mordant k_p'	0.0401	0.0398	0.0323	0.0313	0.00265	**	n.s.			
Rates of digestion (h^{-1})										
Model k_d^*	0.1188	0.0959	0.1231	0.1082	0.00966	n.s.	n.s.	+0.0736	0.00569	***
Dacron bag k_d'	0.0384	0.0333	0.0390	0.0410	0.00270	n.s.	n.s.			
Extent of digestion [g (g NDF intake)$^{-1}$]										
Model P^*	0.697	0.653	0.693	0.682	0.0243	n.s.	n.s.	+0.150	0.0233	***
Dacron bag/mordant P'	0.495	0.482	0.553	0.595	0.0239	**	n.s.			
Estimated from in-vivo digestibilities	0.626	0.606	0.643	0.641	0.0065	***	n.s.	—	—	—

[a] Standard error of mean bias calculated from difference between observations from two methods (31 d.f.).
n.s., Not significant.
Treatments were perennial ryegrass hay offered at two levels of feeding (H and L) with (S) or without maize starch as supplement.

test the assumptions underlying both methods. For digestion, rate constants were obtained by incubation of the hays in dacron bags suspended in the rumen and analysis of the disappearance of fibre from the bags using the Mitscherlich equation of Ørskov and McDonald.[23] The rate constants for passage from the rumen were estimated for hay by fitting the model of Dhanoa *et al.*[24] to the excretion patterns of chromium-mordanted hay, as described by Aitchison *et al.*[21, 22] These estimates were termed k_d' and k_p' respectively.

3.2. Extent of digestion

Apparent digestibility *in vivo* (intake less faeces/intake) was also measured in the same experiments and since the proportion of total fibre digestion which occurs in the rumen does not vary very much[25–28] these values may be used to provide reasonable estimates of the extent of fibre digestion in the rumen against which to evaluate the model. Average values of 0.85 (for grasses) and 0.95 (for legumes) of fibre digestion occurring in the rumen were used to calculate the values in Tables 1 and 2.

The extent of fibre digestion was also predicted from dacron bag and chromium mordant data as given by Ørskov and McDonald.[23] This method was originally developed to calculate the extent of degradation of dietary protein supplements in the rumen but it is reasonable to expect that it should apply to fibre degradation. The extent of digestion, P, is expressed as

$$P=a+bk_d/(k_d+k_p) \tag{12}$$

where a=the fraction of the diet assumed to be instantaneously degraded (the wash value), and b=the fraction of the diet which disappears at a constant rate k_d per unit time.

An estimate thus obtained was termed P'.

3.3. Statistical procedure

Comparison between model predictions and those obtained by conventional methods was made using the method of mean square prediction error analysis (MSPE). This compares the difference between the 'experimental' (i.e. conventional) and the 'predicted' (i.e. model) results in terms of the bias between the values.[29]

4. Results

The predictions (viz. k_p^*, k_d^* and P^*) obtained by use of the model (i.e. from rumen-emptying studies) are compared with those (viz. k_p', k_d' and P') obtained by conventional means (i.e. incubation of the feeds in dacron bags and excretion of chromium-mordanted hay) in Tables 1 and 2.

4.1. Rates of passage

The estimates of rates of passage, k_p^*, for experiment 1 (Table 1) did not differ significantly between treatments, and this effect was also observed from the results of the mordant studies, k_p'. In experiment 2 (Table 2), however, model estimates of passage did not show any significant effect of treatment while the chromium-mordant results showed significantly higher passage rates at the high level of feeding, but no effect of starch inclusion. For both experiments model estimates of rates of passage were significantly ($P<0.001$) lower than those estimates obtained using the chromium mordant; values for k_p^* ranging between 18–61% of their corresponding k_p' values.

4.2. Rates of digestion

The estimates of fractional rates of digestion k_d^* shown in Tables 1 and 2 gave similar trends between treatments as were observed with the dacron-bag estimates: for experiment 1, the rate of digestion of clover was significantly higher ($P<0.05$) than that of the grasses; for experiment 2, starch reduced the rate of digestion ($P<0.10$) compared with treatments without starch. However, the model predictions were consistently higher than those obtained from incubation in dacron bags; values of k_d' ranging between 20–38% of their corresponding k_d^* values.

4.3. Extent of digestion

Predictions of the extent of digestion of the fibre, using the model (equation 10b), the combination of the dacron bag and chromium-mordant (equation 12), and the in-vivo apparent digestibility data, are shown in Tables 1 and 2. The values predicted by the dacron-bag/chromium-mordant technique were significantly ($P<0.001$) lower than those predicted either by the model or the in-vivo digestibilities. Agreement between the latter two predictions was good, with values predicted by the model ranging from 78–111% of the values derived from overall digestibilities.

5. Discussion

The objectives in developing the model were first to estimate rates of digestion and passage from data obtained by emptying the rumen at specific times after once-daily feeding and secondly to use these estimates to improve our understanding of fibre kinetics in the rumen. The model predictions will be considered first and then the validity of the estimates obtained using conventional techniques will be discussed separately.

5.1. Predictions using model

With the model described here, the estimates of rate of passage were obtained from values for the rumen content of indigestible fibre during the non-feeding period. Qualitative assessment of the results used to derive these values,[21, 22] together with the data of Freer *et al.*[30] and Ulyatt *et al.*,[31] suggests that there is a large outflow of indigestible material from the rumen during the feeding period, followed by an interval when very little digesta appears to be removed by passage. This might indicate that passage out of the rumen within a 24 h feeding cycle does not obey first-order kinetics, although the observed increases in passage during feeding are confounded by other accompanying large inflows into the rumen as a result of the animal feeding. However, the model offers a means by which the deviation from first-order kinetics may be quantified.

The rate of passage values estimated by the model in the form of equation (11) (viz. k_p^*) were used to substitute for k_p in equation (3a), together with the measured values for rumen pool size of INDF prefeeding ($X_1(0)$) and the daily feed intake (F_1). This enabled prediction of the amount of INDF in the rumen at the end of feeding. These predictions were then compared with the values obtained by backward extrapolation to time t_f of the actual data using the fitted equation (equation (11)). In all instances, the estimate of INDF in the rumen at t_f using equation (3a) based on k_p^*, was considerably higher than the estimate obtained by extrapolating from the data. This difference became smaller as the value of k_p substituted into equation (3a) was increased from two to five times the original estimate, and may thus give some support to the hypothesis that the fractional rate of passage and not just the actual outflow during feeding is considerably higher than the value of k_p estimated during the

postfeeding period. This suggests either that more than one rate constant applies within a 24 h period or, that first order kinetics do not apply. However, the timing of the data points available in this study does not permit definition of more than one rate constant since the main periods of outflow appear to be during eating and during the second half of the feeding cycle; neither do the data currently available permit derivation of an alternative to first-order kinetics.

Examination of the mechanisms controlling passage of material out of the rumen provides possible reasons for changes in fractional outflow during the day. Both rumen motility and the frequency of contraction of the reticulo-omasal orifice are important in controlling passage of material out of the rumen.[32] Increased contractions during periods of eating and ruminating have been reported (for example, see reference 33) as well as evidence of negative feedback mechanisms relating large loads of digesta in the intestines to decreased rumen motility.[34]

As the model (equation (4b)) uses the passage rates to derive the digestion rates, any bias in the estimation of the former will also affect the latter. However, whereas the rates of passage estimated by the model were in all cases lower than those obtained using chromium-mordant, the rates of digestion estimated from the model were always higher than those obtained using dacron bags (Tables 1 and 2). If the rates of passage were a true underestimate, then from equation (6), the rates of digestion would also have been underestimated since the extent of digestion calculated by the model was in close agreement with the estimates from in-vivo digestibility, at least for the grass hays. There was a greater discrepancy for the clover hay, but this may simply reflect a greater error in measurement of the digestion of the considerably smaller amount of NDF in the clover (294 g NDF kg^{-1} DM) compared to the grass (average 598 g NDF kg^{-1} DM) hays.

5.2. Predictions using conventional techniques

Estimation of the extent of NDF digestion in the rumen using the Mitscherlich equation of Ørskov and McDonald[23] gave very low values in comparison to those estimated from in-vivo NDF digestibility (Tables 1 and 2). This may be due either to an overestimation of rate of passage with chromium-mordant or an underestimation of rate of digestion using dacron bags. The latter effect could be due to the restrictions on digestion imposed by the method (e.g. the lack of rumination); while the mordanting process could have affected rate of passage estimates through increased density affecting outflow.[35] In addition, the analysis of chromium excretion to estimate rate of passage also assumes first order kinetics which may not be correct as discussed earlier. Thus, while these methods may give relative values which can be used to examine treatment effects, the absolute nature of the values in relation to fibre digestion must be seriously questioned.

6. Conclusions

Use of the model described in this paper to calculate rates of passage from rumen-emptying data suggests that in sheep fed once daily, either more than one rate constant applies within a 24 h period, or that first-order kinetics do not apply. Comparison with estimates of extent of fibre digestion in the rumen obtained using dacron-bag and chromium-mordant techniques indicated that the absolute values for the rate constants thus obtained were not accurate, which highlights a need for further studies of methods and techniques to quantify the rates of passage from and digestion in the rumen.

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Effects of Eight Tillage Treatments on a Tropical Alfisol: Maize Growth and Yield

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The effects of eight methods of preplanting tillage systems were investigated on maize growth and yield for eight consecutive crops grown from 1980 to 1983 on an Alfisol in south-west Nigeria. The tillage systems were: (A) no-till with residue mulch, (B) no-till with chiselling in the row zone to 50 cm depth, (C) mouldboard ploughing followed by (fb) harrowing, (D) disc ploughing fb rotovation, (E) no-till without residue mulch, (F) mouldboard ploughing at the end of rains, (G) mouldboard ploughing fb two harrowings with residue mulch (H) mouldboard ploughing fb two harrowings and ridging. In seasons with infrequent rains and long dry spells, seedling emergence was significantly suppressed on ridged plots (treatment H). The 1500 h soil temperature on the ridged seedbed (H) at 5 cm depth exceeded 40°C, and was about 10°C more than in no-till with mulch (A). The oxygen diffusion rate (ODR) at 10 cm depth was the most in the no-till mulch and the least in the furrow bottoms. The soil moisture reserve was larger in the no-till plots than in the ploughed treatments. Plant height and vigour were adversely affected on ridges in seasons with frequent dry spells. In these seasons, maize grain yield declined with the increasing intensity of mechanical tillage. For the eight seasons, the cumulative benefits in grain yield of all the no-till treatments were 1.76 and 0.32 tonnes ha^{-1} in comparison with ridges (H) and ploughed (C, D, F, G) treatments, respectively. The mean seasonal yield was 3.37 and 1.00 t ha^{-1} for the first and second seasons respectively. The seasonal fluctuations in grain yield were related to rainfall amount and the length of growing season.

Keywords: Soil temperature; no-till farming; mulching; ridges; zonal tillage; oxygen diffusion rate; soil moisture.

1. Introduction

A review of tillage research relating crop response to different methods of seedbed preparation indicates the local specificity of these results leading to seemingly contradictory conclusions.[1] This is partly due to the lack of standard terminology. For example, the 'no-till' is often used interchangeably for (a) seeding in an untilled soil but without crop residue mulch and without chemical weed control, (b) seeding in a manually hoed plot without mulch (after burning), (c) seeding through the previous crop residue mulch but without chemical weed control, and (d) seeding through a chemical or mechanically suppressed mulch of a specifically grown crop cover. The effects on crop will obviously reflect differences in these 'no-till' systems and hence the contradiction and confusion. Crop responses to tillage methods also depend on soil properties, rainfall regime, and the crop grown. It is, therefore, appropriate to investigate the effects of a wide range of tillage methods to determine the most suitable systems needed to alleviate specific soil constraints.

In the humid and subhumid tropics, no-till system with crop residue mulch and with chemical weed control has proven effective for soil and water conservation and for preserving soil organic matter content and soil structure.[1] In the semi-arid West Africa, however, deep ploughing once a year at the end of rains is reportedly a conservatively effective measure[2-6]. In some regions, ridges also seemingly improve water conservation and thereby enhance crop growth.[7-9] The effects of a wide

range of different tillage systems on crop growth, however, have not been widely evaluated in different regions of humid and subhumid tropical Africa. The objective of this investigation therefore, was to evaluate the effects of a range of methods of seedbed preparation on soil physical properties and on maize grain yield. The results are discussed in terms of the applicability of various tillage methods for different soils and regions.

2. Experimental

2.1. Site description

These experiments with eight consecutive crops of maize were conducted at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria from 1980 to 1983. IITA is located approximately 30 km south of the northern limit of the lowland rainforest. The total annual rainfall of 900–1300 mm is distributed over two distinct growing seasons: the first from April to July and the second from August to November. The mean rainfall for each season is about 800 and 400 mm, respectively. The soil of the experimental site belongs to Ibadan series,[10] and is classified as clayey, skeletal, isohyperthermic, kaolinitic Oxic Paleustalf. The soil is sandy near the surface and contains a gravelly horizon comprising quartz gravels and iron and manganese concretions between 20 and 80 cm below the surface. The soil has about 3 to 5 cm of available water reserves in the upper 50 cm of soil and has an effective rooting depth of 25 to 30 cm. The vegetation was cleared manually in 1980. The 5 years of regrowth and fallowing previous to 1980 resulted in improvements in soil structure and soil organic matter content.

2.2. Treatments and statistical design

There were eight tillage treatments: A, No-till with previous-season crop residue retained as mulch. B, No-till with previous-season crop residue mulch and chiselling in the row zone to about 50 cm depth once a year. C, Mouldboard ploughing followed by (fb) two harrowings (residue ploughed under). D, Disc ploughing fb rotovation (residue ploughed under). E, No-till with the crop residue removed. F, Mouldboard ploughing in the dry season (during January or February), two harrowings just before seeding (residue ploughed under). G, Mouldboard ploughing fb two harrowings, and the crop residue replaced on the surface as mulch. H, Mouldboard ploughing (residue ploughed under) fb two harrowings, and contour ridging. Ridges were made manually, 75 cm apart and about 30 cm high.

All ploughing, discing, rotovation, harrowing and chiselling operations were done with tractor driven equipment. All no-till plots were sprayed with paraquat (1, 1′dimethyl 4, 4′bipyridilium ion) at 0.5 kg ha^{-1} a.i. Seeding was done manually with a jab planter at 75 cm between and 25 cm within row spacing. There were three blocks of eight plots each. Treatments within the block were assigned at random. The individual plot size was 10×10 m with enough margins on both sides for a tractor to

Table 1. Seedling emergence of maize as affected by different methods of seedbed preparation during the first season 1983 crop

Treatment	Seedling emergence (count 10 m row^{-1})	Mean soil temperature (°C) at 5 cm depth	
		0800 h	1500 h
A	28	29.9	34.1
B	34	28.9	34.9
C	31	29.6	42.1
D	29	29.4	42.7
E	20	29.3	37.9
F	28	29.7	43.7
G	31	30.5	37.3
H	6	28.3	43.9
l.s.d. (0.05)	8	0.8	1.4

DAS=days after seeding.
Mean soil temperature for 25 to 27 April, 1983.

turn without entering into an adjacent plot. All plots received uniform application of fertiliser i.e. 100 kg N, 30 kg P and 30 kg K ha^{-1}. Nitrogen application was made in two split applications of 40 kg ha^{-1} at seeding and 60 kg ha^{-1} 4 weeks later. All plots were weeded manually about 4 to 6 weeks after seeding.

2.3. Observations and analytical methods

Observations were made of crop emergence and soil temperature. The latter was measured at 5 cm using bent-stem mercury-in-glass thermometers. Weekly measurements were made for plant height. Soil moisture content during crop growth was measured gravimetrically for 0–10 cm depth. The oxygen diffusion rate (ODR) of soils was measured with platinum microelectrodes in the row and in the interrow zone at 15 cm depth. For treatments with crop residue mulch, ODR was also measured in the mulched and unmulched zones. The ODR measurements for ridged treatments were made in the furrow and on the ridge top. Grain yield and yield components were measured at harvest.

3. Results and discussion

3.1. Seedling emergence and crop establishment

With good rainfall and favourable distribution, crop establishment was generally uniform and satisfactory in all treatments. In seasons when a 3 to 4-week long drought followed immediately after seeding, crop establishment was significantly affected by treatments. For example, with a 3-week rainless period in April 1983, crop establishment in the ridged plots was patchy, emergence lower than in the other treatments (Table 1) and reseeding was necessary. The removal of crop residue mulch from no-till plot (comparison among treatments A and E) also resulted in poor crop establishment when drought followed seeding. Replacement of the residue mulch after ploughing and chiselling treatments slightly improved seedling emergence (comparison between treatments C and G, and A and B). Differences in crop establishment between treatments when drought followed seeding are attributed to differences in soil temperature and moisture regimes and in surface crusting.

There were no significant differences in soil temperature at 5 cm depth measured at 0800 h. There were, however, significant differences in the maximum soil temperature measured at 5 cm depth at 1500 h during this drought period (Table 1). At 1500 h, differences in soil temperature of about 10°C between no-till with mulch (A) and ridged treatments were not uncommon. The residue removal from no-till plots (comparison among treatments A and E) resulted in an increase of 3 to 4°C in soil temperature. Mulching a ploughed treatment decreased the 1500 h soil temperature by 4 to 5°C (comparison between A and G). Similar effects of mulching, no-till, and ridging have been reported before.[11, 12] Diurnal fluctuations in soil temperature between different treatments from 18 April and 11 May 1983 are shown in Figure 1. Because only ploughed unmulched plots developed surface crust, maize emergence was affected by the interaction of crust strength with the soil temperature and moisture regimes. The crusting was not severe in unmulched treatments.

3.2. Oxygen diffusion rate

Crust development also influences gaseous exchange between the soil and the atmosphere. The data in Table 2 compare the oxygen diffusion rates among treatments observed at different growth stages of the 1983 first season crop. In general, the ODR was high for no-till treatments. Immediately after heavy rain on 47 DAS, the least ODR was measured in rotovated and ridged treatments and the maximum in chiselled plots (Table 2). Both ridged and rotovated treatments developed thick surface crusts. These soils are generally well drained, and therefore, an inhibited gaseous exchange is often not a severe limiting factor in upland soils.

There were local variations in ODR due to the presence of crop residue mulch. In the no-till plots (A), the ODR measured 20 DAS in the mulched interrow zone and the unmulched row zone was 0.520 ± 0.190 and 0.313 ± 0.174 $\mu g\ cm^{-2}\ min^{-1}$ in Block 1, 0.426 ± 0.175 and 0.266 ± 0.095 in Block 2,

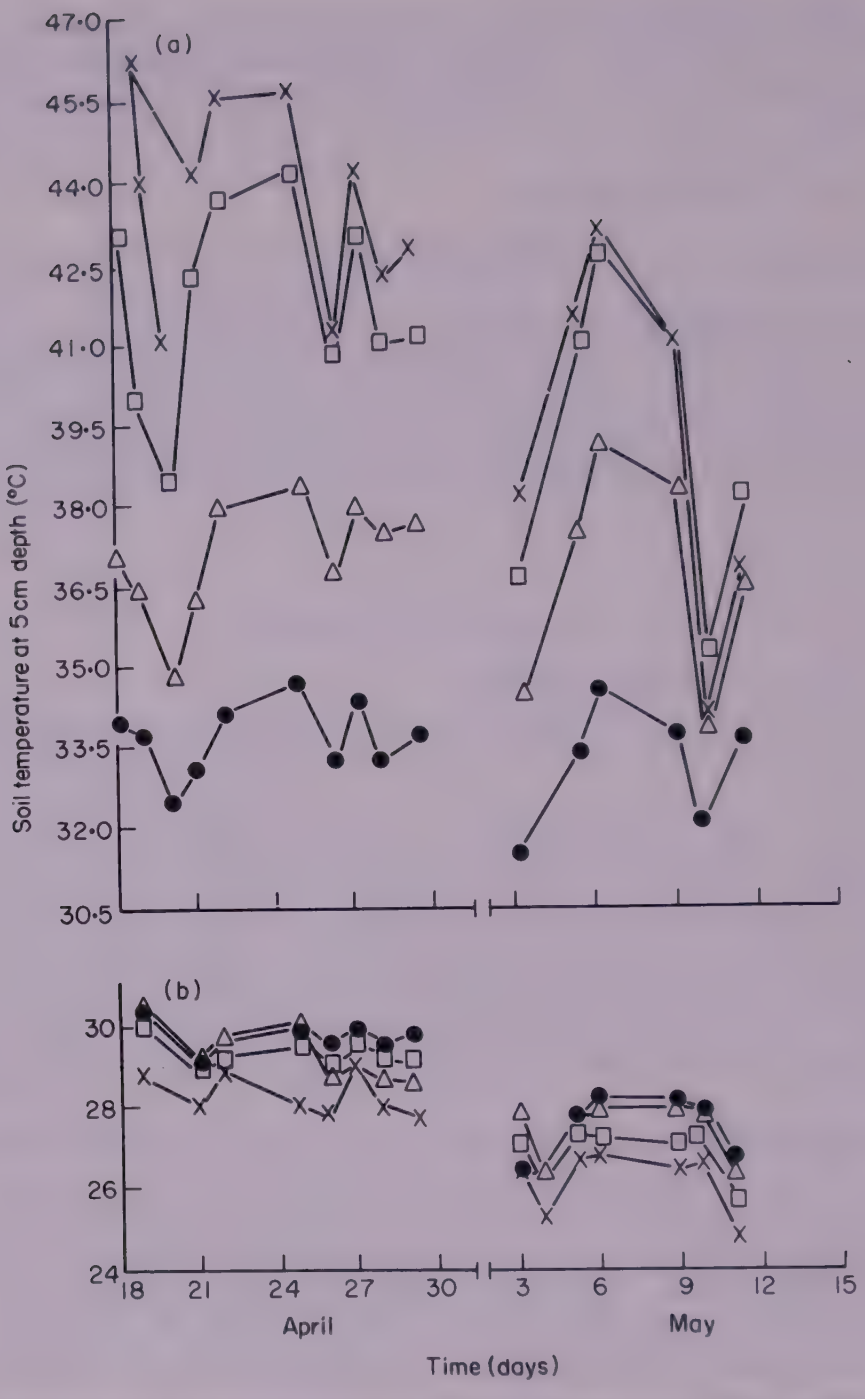

Figure 1. Diurnal fluctuations in soil temperature for selected tillage treatments at (a), 1500 h and (b), 0800 h: (×), ridges; (●), no-till with mulch; (□), ploughed without mulch; (△), no-fill without mulch.

and 0.601±0.0189 and 0.423±0.213 in Block 3, respectively. In ridged plots, ODR was considerably reduced in the furrows compared to that on ridge top. There were considerable variations in ODR among blocks. The soils of block 1 have 2–3% more clay and 2–3% more silt than that of Block 3; and are more prone to crusting. Consequently there are appreciable differences in ODR between treatments in Block 1 than in Block 3. The ODR measured in ridge top and furrow was 0.695±0.130 and 0.421±0.204 $\mu g\ cm^{-2}\ min^{-1}$ in Block 1, 0.739±0.043 and 0.643±0.016 in Block 2, and 0.756±0.019 and 0.746±0.008 in Block 3, respectively.

3.3. Soil moisture content

During dry spells, maize grown in ridged (H) and all ploughed treatments (C, D, F, G) experienced severe drought. The soil moisture content at both 0–10 cm depth was always low in ridged and all ploughed treatments. The data on soil moisture at 0–10 cm depth for the second season 1980 crop are

Table 2. Effects of tillage methods on oxygen diffusion rate ($\mu g\ cm^{-2}\ min^{-1}$) at 15 cm depth during the first season 1983 crop

Treatments	Days after seeding			
	21	26	32	42
A	0.516a	0.652ab	0.722a	0.682ab
B	0.599a	0.530b	0.674a	0.630ab
C	0.458a	0.604ab	0.637ab	0.653ab
D	0.568a	0.588ab	0.696a	0.719a
E	0.441a	0.630ab	0.720a	0.665ab
F	0.521a	0.577ab	0.565b	0.592ab
G	0.568a	0.629ab	0.703a	0.666ab
H	0.583a	0.637ab	0.704a	0.530b
l.s.d. (0.05)	0.193	0.138	0.099	0.138

Figures in columns followed by same letters are statistically similar.

shown in Table 3. There were considerable variations in soil moisture content probably due to spatial variations in gravel content.

The data show consistently lower soil moisture reserves on ridged (measured on ridge top) plots throughout the growing season. Comparing the soil moisture reserves of ridged with the mean moisture content of no-till treatments (A, B, E), ridged plots had lower moisture content by 20.6,

Table 3. Effects of tillage methods on soil moisture content (%, by wt) during the second season, 1980 crop (0–5 cm depth)

Tillage treatments	Days after seeding				
	30	44	51	58	67
A	14.6a	11.5a	15.2a	15.4ab	7.3b
B	15.2a	10.1a	17.7a	16.2ab	12.2a
C	15.0a	8.5a	13.2a	14.3ab	11.0ab
D	13.3a	9.3a	14.4a	15.6ab	13.6a
E	12.4a	10.2a	15.4a	17.5a	12.7ab
F	15.3a	8.7a	13.3a	15.7ab	14.5a
G	14.0a	9.8a	14.2a	13.6ab	8.4ab
H	11.2a	9.7a	12.3a	10.9b	10.0ab
l.s.d. (0.05)	4.6	6.0	6.8	3.7	5.4

Figures in columns followed by the same letters are statistically similar.

Table 4. Effects of tillage methods on maximum plant height (cm) at about 40 DAS (A) and at initial tasseling stage (B) for the 1983 crop in the first season.

Treatment	1983	
	A	B
A	60b	258ab
B	74a	254ab
C	57b	267a
D	62b	263ab
E	59b	224bc
F	63b	258ab
G	59b	264ab
H	41c	215c
l.s.d. (0.05)	11	36

Figures in columns followed by the same letters are statistically similar.

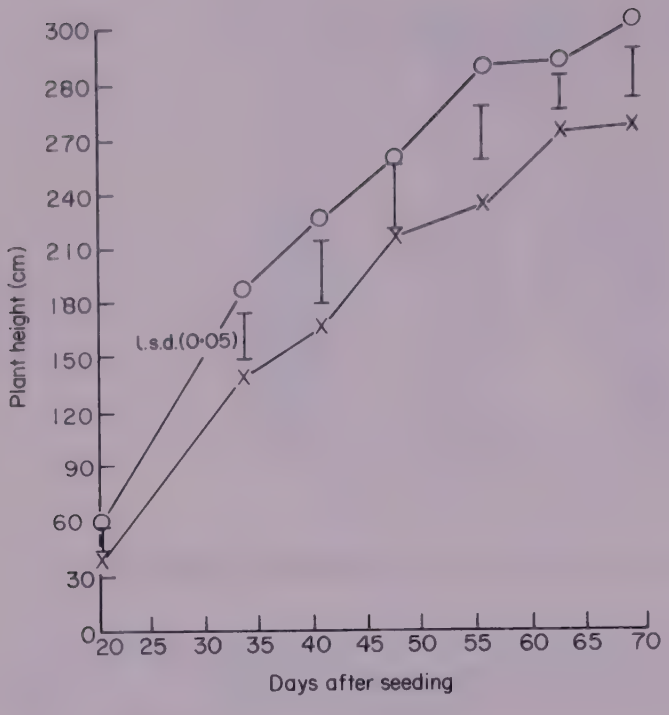

Figure 2. Plant height on ridged and non-ridged treatments: (○), no-till with mulch; (×), ridged.

8.5, 23.6, 33.5, 7.1 and 6.5% for measurements made on 26, 40, 47, 54, 63 and 75 DAS, respectively. In comparison with ploughed treatments (C, F, G), ridged treatments had different moisture content by −24.3, +7.8, −9.6, −24.8, −7.9 and −11.5% for different DAS, respectively. In comparison with ploughing (C, F, G), discing and rotovation (D) treatments had favourable moisture contents. Chiselling of no-till plots (B) improved soil moisture content in comparison with no-till with or without mulch (A and E) by +12.6, −7.3, +15.7, −1.8, +56.8 and 22% at different DAS respectively.

In general the moisture content was high in all no-till plots, and low in ridged treatment. Ploughing caused no definite improvements in soil moisture reserves. Even ploughing in the dry season (F) showed no consistent improvements in soil moisture reserves over ploughed or disced plots (C, D, G). The residue removal in the no-till treatment had also no deleterious effects on soil moisture reserves (Table 3).

3.4. Plant growth and vigour

Plant growth was not different among treatments in seasons with adequate and favourably distributed rains. During these seasons, plant height on ridged treatments was equal to or better than

Table 5. Maize grain yield (Mg ha ^{1}) for eight different seasons as influenced by different methods of seedbed preparation

	1980		1981		1982		1983	
Treatment	I	II	I	II	I	II	I	II[a]
A	2.5a	2.4a	3.6a	0.8bc	4.6a	1.2a	4.4a	0.5c
B	2.0a	1.8a	3.4a	1.0abc	4.3ab	0.9a	4.2a	1.4b
C	2.7a	2.0a	3.1ab	1.3ab	3.8ab	1.1a	3.7a	2.3a
D	2.4a	2.1a	3.4a	1.0abc	3.8ab	0.8a	4.6a	1.8ab
E	2.1a	1.8a	2.8ab	0.8bc	4.2ab	1.0a	3.6a	1.8ab
F	2.6a	1.8a	3.4a	0.7c	3.8ab	0.9a	3.8a	2.1ab
G	2.1a	2.0a	2.9ab	1.0abc	3.9ab	1.0a	4.4a	1.6b
H	2.5a	2.1a	2.2b	1.4a	3.7b	0.8a	3.4a	1.3bc
l.s.d. (0.05)	0.8	1.0	1.2	0.5	0.7	0.4	1.6	0.8

[a]Stover yield only.

I, First season; II, second season.

Figures in columns followed by the same letters are statistically similar.

other treatments (Table 4). This was particularly so during both seasons of 1980 and 1981. Drought and severe soil moisture stress experienced during 1982 and 1983 seasons adversely affected maize growth and vigour in ridged treatment. In addition to delayed emergence, seedling growth was stunted. Maize leaves in ridged plots showed chlorotic symptoms, tip burning and interveinal chlorosis. Similar symptoms were reported by Maurya and Lal[12] in maize plants mulched with clear plastic mulch where soil temperature exceeded 45°C. Maize seedlings suppressed by supra-optimal soil temperature in ridged treatment never recovered and their height was significantly lower than in no-till mulched plots throughout the season (Figure 2, Table 4).

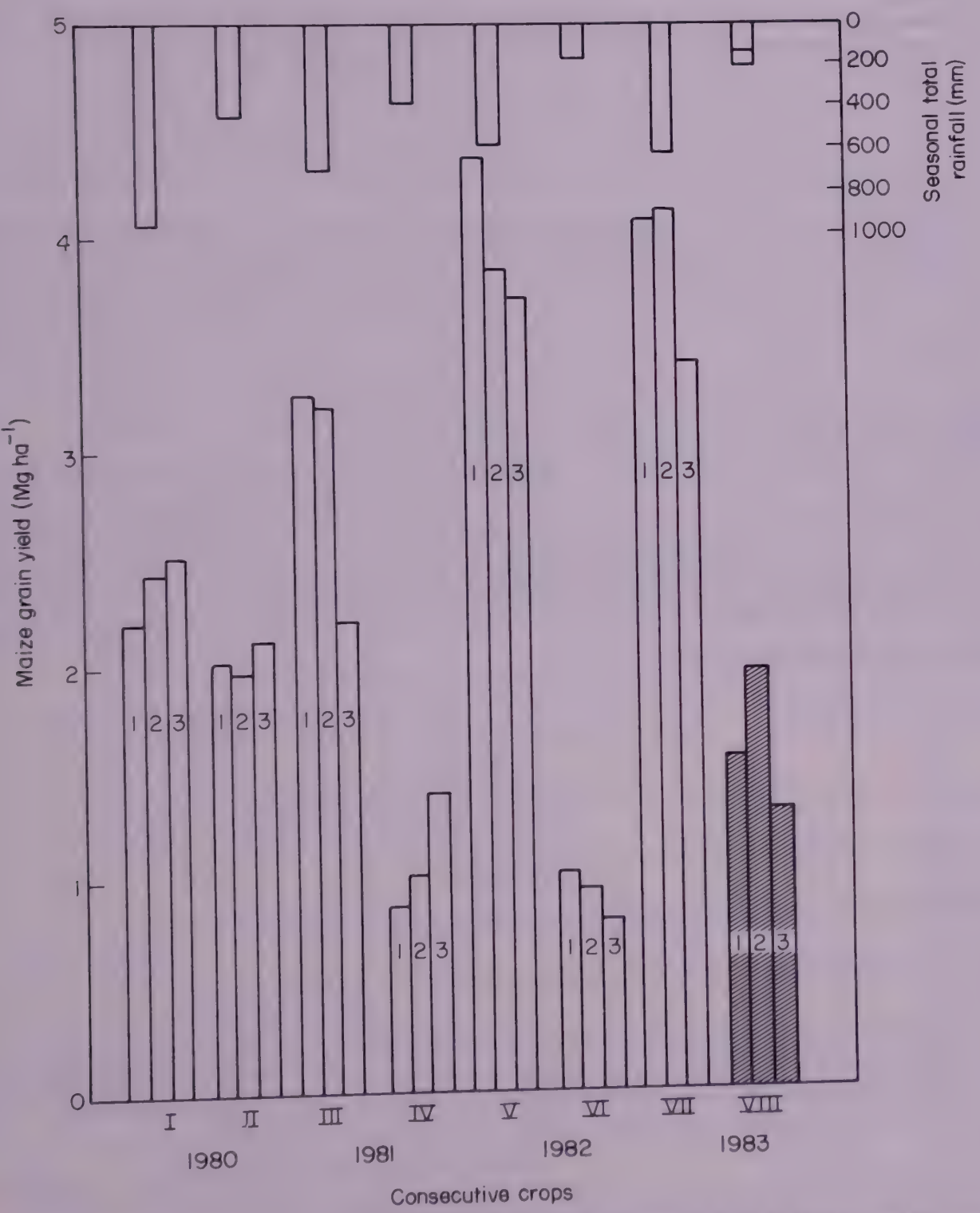

Table 3. Comparison of maize grain yield for 1, no-till (A, B, E) with 2, ploughed (C, D, F, G,) and 3, ridged (H) treatments: (▨), stover yield only (no grains).

In seasons with good rains, there were either no differences in plant height among various treatments or the maize in ploughed plots was slightly superior to those in no-till treatment. During the first season in 1980, the maximum plant height and vigorous growth was observed for the ploughed treatment C, and the least for rotovated plot in treatment D. For the second season in 1980, the least height and vigour were observed in no-till plots without crop residue mulch. There were no measurable differences in plant growth among treatments for the 1981 crop in the first season, while in the second season the least height and vigour were observed in the treatment with ploughing in the dry season.

3.5. Grain and stover yield

The maize grain yields for eight consecutive crops are shown in Table 5. Like plant height maize grain yield was not affected by the tillage treatment in either crop of 1980. In 1981, the grain yield in ridged plots was the least in the first season and the most in the second season. For both crops in 1982, the maize grain yield in ridged plots was the least and was significantly lower than no-till with mulch. For the first season crop in 1983, there were no significant differences in maize grain yield among treatments although the least yield was once again recorded for the ridged plots. There was no grain yield for the second crop in 1983 as abrupt cessation of rains about 7 weeks after seeding resulted in the crop failure.

Comparison of maize grain yield for no-till (mean of treatments A, B and E) with ploughed (treatments C, D, F and G) and ridged (treatment H) seedbeds is shown in Figure 3. The mean grain yield (averaged over the eight seasons) was 2.0, 2.1 and 2.2 Mg ha^{-1} $season^{-1}$ for ridged, ploughed and no-till treatments. The differences in grain yield between treatments are small and statistically non-significant. However, the trends in grain yields over the eight seasons were in favour of no-till treatments. For the eight seasons, the cumulative benefits in grain yield by the no-till system were 1.8 and 0.3 Mg ha^{-1} in comparison with ridges and ploughed systems, respectively. In general, the grain yield decreased with an increasing intensity of mechanical tillage.

The mean seasonal yield (averaged over all treatments) was 3.4 and 1.0 Mg ha^{-1} for the first and the second seasons, respectively. The low yield in the second season was related to the low total amount of rainfall and to the shorter length of growing season. Maize grain yield over eight seasons was positively highly correlated (R^2=0.76) with the total amount of rains received. There was a quadratic relationship between grain yield and the seasonal accumulative rainfall. The maximum yield occurred at a mean seasonal rainfall of 740 mm. A rainfall of only 140 mm in the second season resulted in zero grain yield (Figure 4).

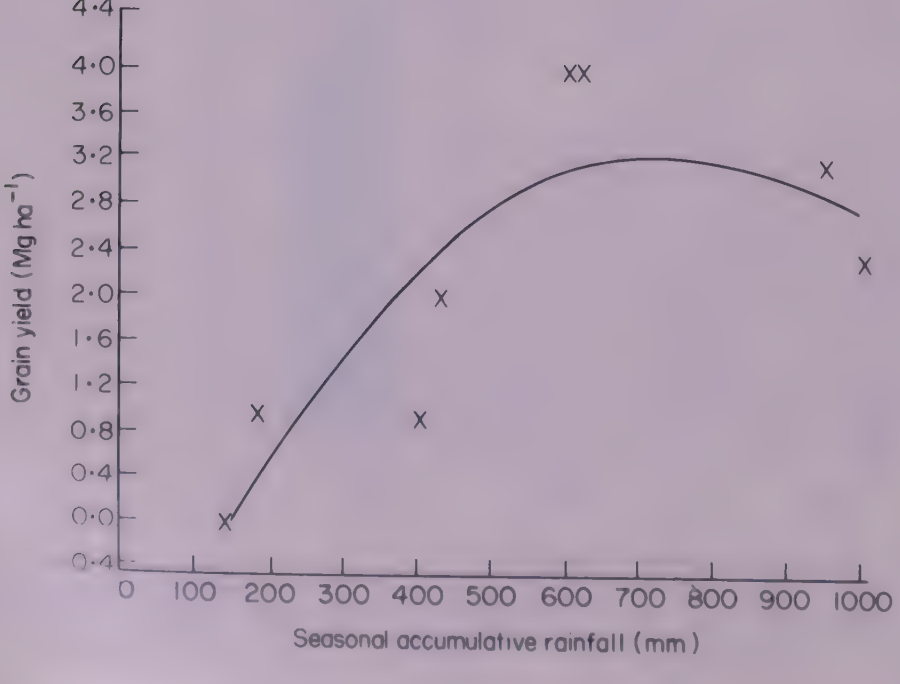

Figure 4. Relationship between maize grain yield in all eight seasons and the accumulative seasonal rainfall:

Fitted curve
$y=-1.84+0.0143\,x-0.0000097x^2$,
$R^2=0.76$.

Table 6. Maize stover yield (Mg ha^{-1}) for the first seasons in 3 years influenced by different methods of seedbed preparation

Treatment	1981	1982	1983
A	3.0a	4.7a	4.4ab
B	2.9ab	4.2ab	4.2ab
C	3.0a	4.5a	5.5a
D	3.3a	4.2ab	5.2ab
E	2.2b	4.7a	3.6ab
F	3.0a	3.4b	4.5b
G	2.2a	4.5a	4.3ab
H	2.0b	4.0ab	3.2b
l.s.d. (0.05)	1.0	0.8	1.8

Figures in columns followed by the same letters are statistically similar.

The data in Table 6 show the effects of various tillage treatments on stover yield for the first season crops from 1981 to 1983. In two out of three crops, the least stover yield was obtained for ridged plots. The removal of residue mulch from no-till plots also suppressed stover yield and there was no improvement in stover yield by chiselling of no-till plots. Application of crop residue mulch following ploughing improved maize stover yield in 1 out of 3 years.

4. General discussion and conclusions

The seasonal differences in grain yield indicate that maize is not a suitable crop for the short second season in this region. Considering all factors involved, a short duration cowpea would be a better suited crop for the second season than maize.

Maize grain yield was either unaffected or slightly decreased with the increasing intensity of mechanical tillage operations. The data support the following conclusions:

(a) For seasons with adequate and equitable distribution of rains, maize yields in ploughed or ridged treatments were equivalent to or only slightly better than in no-till treatments with or without residue mulch and with or without chiselling.

(b) For seasons with long dry spells and inadequate rains, ridges yielded the lowest grain yield, and maize yields in no-till systems were equivalent to or better than mechanically tilled treatments.

(c) Removal of crop residue mulch from no-till system often resulted in a decrease in grain and stover yields, although the adverse effects were only slight.

(d) Chiselling of no-till plots had no beneficial effect on grain or stover yield for this coarse-textured soil newly developed after a long bush fallow rotation.

(e) Replacing the crop residue mulch following ploughing was only slightly beneficial to maize grain and stover yield.

(f) High soil temperatures can be a limiting factor towards seedling establishment in ridged and ploughed seedbed.

(g) These soils are well drained and the reduced gaseous exchange is not a constraint to maize even during the periods of frequent rains.

From a series of experiments already reported for this ecological environment (with predominantly Alfisols of medium fertility, having a coarse textured surface horizon and susceptible to erosion and drought), there are no beneficial effects of mechanical tillage over no-tillage or reduced tillage systems.[1] Chiselling in the row zone may have an ameliorative effect if the soils are initially compacted as often happens in mechanised farming.[13, 14]

Ridges are often used as a conventional method of seedbed preparation. The data from this and previous experiments[11] show either the negative or no beneficial effects of ridges over no-till or flat planting system. Whatever the beneficial effects of tied ridges in soil and water conservation may be

in soils of semi-arid regions,[7–9, 15] contour ridging is not an effective soil conservation technique for the sub-humid West Africa. The adverse effects of ridges in terms of high soil temperature and low soil moisture outweigh the doubtful utility of this practice in soil and water conservation. In this ecological environment the most useful tillage system for grain crops combines the provision of crop residue mulch on the soil surface and the elimination of all mechanical tillage before planting.

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The Effects of Defaunation on the Metabolism of Lactic Acid in the Rumen

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Two experiments examined the effects of defaunation on the ruminal metabolism of lactate. Three rumen-cannulated sheep given a diet of molassed sugar beet pulp and barley (80:20) were used to study the effects of defaunation (with manoxol-OT) on the metabolism of lactic acid produced endogenously from rumen fermentation. Defaunation increased mean ruminal concentrations of lactate from 3.4 mmol litre^{-1} to 8.9 mmol litre^{-1} but other rumen measurements remained virtually unchanged: pH, 6.3 and 6.3; molar proportions of acetic acid, 645 and 645 mmol mol^{-1}; propionic acid, 189 and 197 mmol mol^{-1} and butyric acid 142 and 115 mmol mol^{-1} for the faunated and defaunated states respectively. In a second experiment, two groups each of four rumen-cannulated sheep were used to study the effect of defaunation on the ruminal metabolism of added Na-D, L lactate. One group of four was defaunated using a rumen-washing technique whilst the other group of four remained faunated. The animals were given a diet of rolled barley and hay (60:40) and were given a series of intraruminal doses of Na-D, L lactate from 0 to 100 g day^{-1}, increasing by 20 g day^{-1} every third day. Disappearance of added lactate was much more rapid in faunated animals: at the 100 g day^{-1} dose rate, L-lactate concentrations had fallen from a peak of 6 g litre^{-1} to <1 g litre^{-1} after 3 h whereas in defaunated sheep the peak of 6.5 g litre^{-1} was reduced to <1 g litre^{-1} only after 7 h. In faunated animals lactate addition caused an increase in the ruminal concentration of total volatile fatty acids (VFA) from 147 to 217 mmol litre^{-1} between the zero and 100 g day^{-1} dose rates, accompanied by an increase in the molar proportion of propionic acid from 190 to 320 mmol mol^{-1}. However, in defaunated animals there was only a small increase in total VFA concentrations from 94 to 106 mmol ml^{-1} with no change in the molar proportion of propionic acid and a small increase in butryric acid from 140 to 180 mmol mol^{-1}.

Keywords: Rumen; lactic acid; defaunation; protozoa.

1. Introduction

Normally, ruminal concentrations of lactic acid remain low since the rates of production and utilisation of lactate by rumen microorganisms are well balanced. However, in some dietary circumstances an unbalanced fermentation can occur, leading to an accumulation of lactic acid with possible deleterious effects on animal health and production.[1]

The microorganisms traditionally associated with the utilisation of lactic acid in the rumen are the bacteria.[2–4] However, recent studies on the ruminal digestion of grass silage diets in sheep and cows have implicated the protozoa in lactic acid metabolism.[5, 6] This raises the question whether protozoa play a more general role in the ruminal metabolism of lactate, in particular whether they make an important contribution to the utilisation of lactate arising endogenously from rumen fermentation. The first experiment reported here examined the effects of defaunation on the metabolism of lactic acid arising endogenously from rumen fermentation of a diet of molassed sugar beet pulp and barley. A second experiment is also reported, the objective of which was to determine to what extent the

absence of protozoa might impair the capacity of the rumen to metabolise lactic acid entering the rumen exogenously, as would be the case with a silage diet. Parts of this work have already been reported briefly.[7, 8]

2. Experimental

2.1. Animals and their management

A total of 12 mature wether sheep weighing between 60 and 70 kg were used in the two experiments. The animals were each prepared with a permanent cannula into the rumen and were housed in metabolism cages for the duration of the experiment. Diets were fed in two equal meals at 0900 hours and 1600 hours; water and mineralised salt licks were freely available at all times.

2.1.1. Experiment 1

Three sheep were adapted to a diet of sugar beet pulp (800 g day^{-1}) and hay (200 g day^{-1}) (Table 1) over a period of 14 days before the start of sampling. Samples (50 ml) of rumen fluid were withdrawn from the rumen at 0, 0.5, 1, 1.5, 2, 3 and 5 h after the morning feed (period 1, faunated). The animals were then defaunated by the addition of manoxol-OT[9] (feed was withdrawn 36 h before the addition of 5 g manoxol-OT in 200 ml water via the rumen cannula on two consecutive days) and on the fifth day after the last manoxol addition, rumen samples were withdrawn as above (period 2, defaunated). Following sampling, animals were allowed to refaunate (refaunation was encouraged by the intraruminal addition of 1 litre of rumen fluid from a faunated donor sheep on a similar diet) and after 14 days rumen samples were withdrawn as described above (period 3, refaunated). Defaunated sheep were isolated from other animals by a distance of not less than 3 m.

Table 1. The composition of components of the diets used in experiments (g kg DM^{-1} except where stated)

	Hay experiment 1	Sugar beet pulp	Hay experiment 2	Barley
Dry matter (g kg^{-1})	852	869	853	847
Ash	62	103	73	23
Total nitrogen	13.3	16.9	10.8	15.8
Acid detergent fibre	434	163	374	54

2.1.2. Experiment 2

Two groups of animals were prepared as follows. Group 1, four sheep (faunated) in metabolism cages in contact with other animals; group 2, four sheep, defaunated by physical emptying of the rumen followed by rinsing with warm tap water on three consecutive days,[10] in isolated metabolism cages, fed and managed separately from the faunated animals.

Animals were adapted to a diet of barley (600 g day^{-1}) and hay (400 g day^{-1}) (Table 1) over a period of 14 days before the start of sampling. During the experimental period increasing levels of Na D, L-lactate were added to the rumen, with the dose being increased by 20 g day^{-1} on every third day Na D, L-lactate syrup was diluted to the appropriate concentration in 500 ml of tap water and quickly added to the rumen, via the rumen cannula, immediately before the morning feed. Rumen samples were withdrawn at 0 h (prefeed and dosing), 0.5, 1, 1.5, 2, 3, 5 and 7 h after the morning feed on all days of treatment for both groups of animals.

2.2. Analytical methods

Samples of rumen fluid were strained through a double layer of cheesecloth and the pH taken immediately. Samples were preserved for further analysis of ammonia, volatile fatty acids and D and L lactate concentrations.[11-13] Protozoal numbers were determined at each sampling time in experiment 1 and at 2 h post feed in experiment 2.[14] Numbers of total culturable and lactate-utilising

Table 2. The effects of defaunation on the pH, composition of rumen fluid and on protozoal and bacterial counts in sheep fed sugar beet pulp and hay

	Faunated	Defaunated	Refaunated	s.e.d.
pH	6.3	6.3	6.3	0.14
Ammonia nitrogen (mg litre^{-1})	62	11	84	5***
Total VFA (mmol litre^{-1})	80.4	73.7	93.9	12.1
Individual VFAs (mmol mol^{-1} total VFA)				
Acetate	645	648	627	20.2
Propionate	189	197	154	28.3
Butyrate	142	115	204	15.0**
Branched-chain[a]	22	46	19	1.1**
L-lactic acid (mmol litre^{-1})	3.4	8.9	7.6	1.4**
Protozoa ($\times 10^5$ ml^{-1})	3.3		5.1	0.5**
Total culturable bacteria ($\times 10^{10}$ ml^{-1})	9.5	171	97	22.3**
Lactate utilising bacteria ($\times 10^{10}$ ml^{-1})	0.5	9.1	3.5	1.6**

[a]Branched-chain acids: isobutyrate, valerate, isovalerate.
$P<0.01$; *<0.001.

bacteria were estimated in strained rumen liquor withdrawn 2 h post feed in experiment 1 and 5 h post feed in experiment 2.[15] Methods for the analysis of foods have been described.[16]

2.3. Statistical results

Results were analysed statistically according to a split plot design in experiment 1 and by a factorial design in experiment 2.[17]

3. Results

3.1. Experiment 1

Results as means over the day are shown in Table 2. No significant differences between treatments were found in rumen pH, total volatile fatty acid (VFA) concentrations or the molar proportions of acetate and propionate in the rumen, but there was a trend towards reduced concentrations of total VFA and slightly increased molar percentages of propionate in defaunated animals. Ruminal concentrations of L-lactate were significantly higher in defaunated animals than in faunated animals. Although refaunation led to lower levels of ruminal lactate than present in defaunated animals the decrease was not significant ($P>0.05$) and values remained significantly higher than in period 1

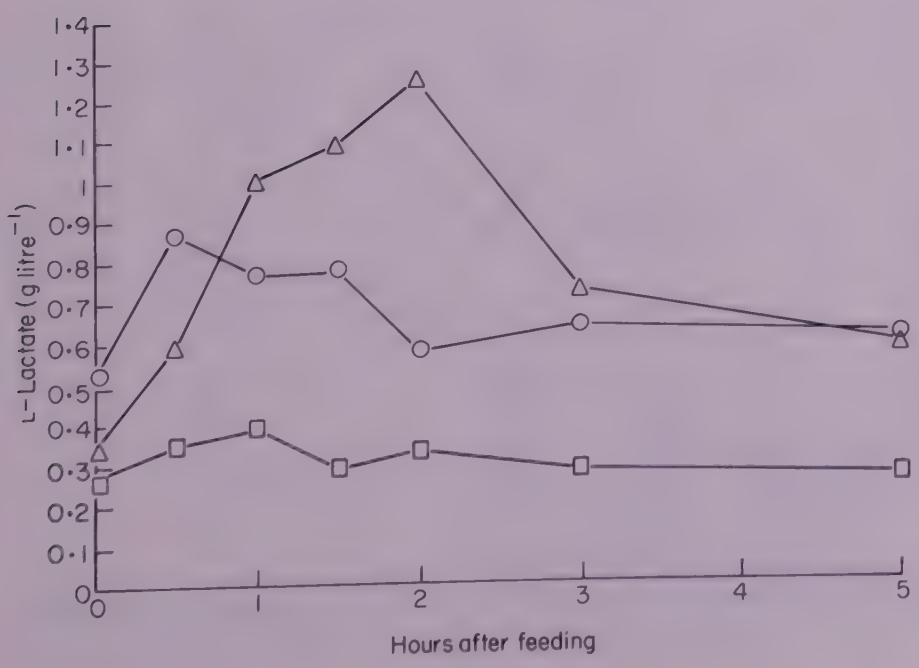

Figure 1. Rumen L-lactate concentrations in: (□), faunated; (△), defaunated and (○), refaunated sheep fed sugar beet pulp and hay.

Table 3. The effects of defaunation on the pH, composition of rumen fluid and protozoal counts in sheep intraruminally dosed with Na D, L-lactate

Na D, L-lactate infused (g day^{-1})	Faunated [Na D, L-lactate infused (g day^{-1})]						Defaunated [Na D, L-lactate infused (g day^{-1})]						
	0	20	40	60	80	100	0	20	40	60	80	100	s.e.d.
L-lactate (mmol litre^{-1})	2.3	4.4	5.9	8.0	20.0	24.3	2.1	9.2	12.2	19.4	24.7	34.4	1.8***
D-lactate (mmol litre^{-1})	2.7	3.8	4.1	8.5	13.9	18.8	1.8	6.2	10.1	13.0	15.7	18.4	1.0***
pH	6.5	6.5	6.6	6.7	6.5	6.6	6.5	6.8	6.8	6.7	6.8	6.8	0.03***
Total VFA (mmol litre^{-1})	147	137	159	194	186	217	94	103	92	90	90	106	6.0***
Individual VFAs (mmol mol^{-1} total VFA)													
Acetate	660	620	590	570	560	570	610	620	630	610	610	580	0.8***
Propionate	190	230	250	290	310	320	220	200	200	210	200	220	0.6***
Butyrate	130	130	130	120	110	100	140	170	140	150	170	180	0.6***
Protozoal numbers ($\times 10^5$ ml^{-1})	10.9	6.6	6.9	7.4	5.2	6.5	—	—	—	—	—	—	0.8*

*$P<0.05$; ***$P<0.001$.

($P<0.01$). The pattern of change in L-lactate concentrations over the day also varied with treatment (Figure 1). In defaunated animals levels rose to a peak in the first 2 h after feeding and remained elevated over the rest of the sampling period. In the faunated animals (periods 1 and 3), however, lactate concentrations rose to a peak within an hour after feeding and quickly fell to a steady level.

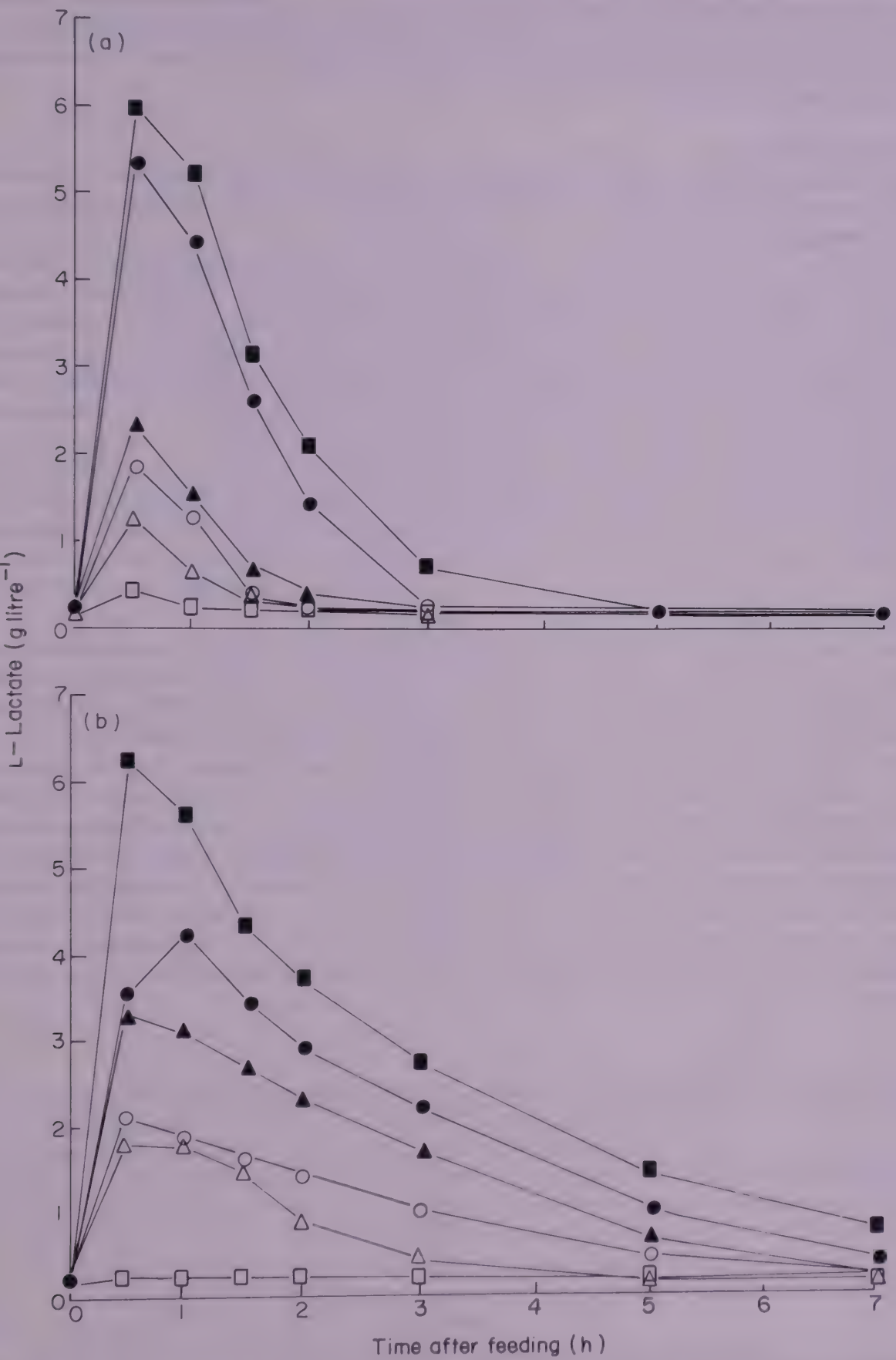

Figure 2. Rumen L-lactate concentrations in (a) faunated and (b) defaunated sheep following intraruminal dosing with (□), 0; (△), 20; (○), 40; (▲), 60; (●), 80; and (■) 100 g day^{-1} Na D, L-lactate.

The molar proportion of total VFA present as butyrate was slightly reduced following defaunation, while levels were considerably increased in the refaunated animals ($P<0.01$). Concentrations of branched-chain acids were elevated in defaunated sheep while rumen ammonia levels were reduced.

Numbers of lactate-utilising and total culturable bacteria were significantly raised during defaunation and although numbers fell on refaunation they did not reach values comparable to period 1. The percentage of the culturable bacteria present as lactate utilisers did not vary with treatment and remained constant at around 5% of the total.

3.2. Experiment 2

Rumen pH was significantly higher in defaunated sheep over the whole experiment, but no discernible trends in the mean daily pH values of either group were noticeable with increasing doses of Na D, L-lactate (Table 3).

When Na D, L-lactate was added to the rumen, mean daily concentrations of L-lactate were significantly higher in defaunated animals (Table 3) mainly owing to the slower rate of removal of added lactate from the rumen of these animals (Figure 2). Results for D-lactate were similar. Rumen D and L-lactate concentrations in defaunated sheep rose in a linear fashion when increasing amounts of Na D, L-lactate were added to the rumen; in faunated animals the response was more curvilinear, with rumen levels of D and L-lactate rising less rapidly when low levels of Na D, L-lactate were added to the rumen than when higher levels (>60 g day^{-1}) were used (Table 3).

Total VFA levels were significantly lower in defaunated sheep and whereas total VFA concentrations after feeding were considerably increased by the addition of Na D, L-lactate in faunated sheep, no such increase was evident in defaunated sheep. Thus in faunated animals, mean concentrations of total VFA during the day tended to increase with increasing additions of Na D, L-lactate to the rumen while in defaunated animals levels were generally lower and did not respond to Na D, L-lactate addition (Table 3).

The molar proportions of the VFA were considerably different between faunated and defaunated animals when no Na D, L-lactate was added to the rumen, with significantly higher levels of propionate and butyrate, and a lower proportion of acetate in defaunated sheep (Table 3). In the faunated group, the molar proportion of VFA present as propionate increased as the amount of Na D, L-lactate added to the rumen increased. However in the defaunated group the proportions of VFA remained more stable, although there was a slight increase in the molar proportion of butyrate.

Total culturable bacteria were significantly higher in defaunated animals (Table 4) with counts approximately seven-fold higher throughout the experiment. Numbers of culturable lactate-utilising bacteria were also higher in defaunated animals, being approximately 12-times higher than those in faunated animals. Bacterial numbers rose to a similar degree in both groups of sheep when Na D, L-lactate was added to the rumen, and there was no discernible trend in the proportion of lactate-utilising bacteria in the total bacteria as the level of Na D,L-lactate added to the rumen was increased (Table 4).

Table 4. The effects of defaunation on bacterial numbers in the rumen of sheep intraruminally dosed with Na D, L-lactate

Na D, L-lactate (g day^{-1})	Total culturable bacteria (×10^9 ml^{-1})			Culturable lactate utilising bacteria (×10^9 ml^{-1})		
	F[a]	DF	s.e.d.	F	DF	s.e.d.
0	41	199	52**	1.6	17.0	7.5
20	79	539	92**	2.7	21.6	3.9**
40	98	686	95**	1.2	27.3	7.4*
60	247	2423	1077	11.0	65.0	20.1*
80	724	4787	1084**	23.6	252.5	76.7**
100	844	4186	1077**	18.5	298.3	33.0**

[a]F, faunated; DF, defaunated.
*$P<0.05$; **$P<0.01$.

4. Discussion

Complete defaunation using manoxol was difficult to achieve and several attempts were frequently necessary. Several combinations of increased doses (10 g) and repeated additions of manoxol were tested but since high levels of manoxol (>5 g day^{-1}) had undesirable effects on the sheep, a lower level (5 g day^{-1}) was used to defaunate sheep in experiment 1. As defaunation was difficult to obtain and maintain with manoxol, experimental observations had to be made on the fifth day after its administration. On the other hand, defaunation by rumen washing[10] proved easy and reliable with animals remaining protozoa-free for up to 6 months.

The overall aim of the experiments was to examine the effects of defaunation on ruminal metabolism of lactic acid in dietary circumstances characterised either by an endogenous production of lactic acid from the rumen fermentation or by an exogenous entry of lactic acid, as would occur with diets containing silage. The diet of molassed sugar beet pulp and hay used in experiment 1 was chosen since it was known to be associated with an endogenous production of lactic acid as judged from the moderately high concentrations of lactic acid in rumen fluid.[6]

In experiment 1 defaunation resulted in elevated ruminal concentrations of lactic acid, an observation noted previously.[18, 19] The higher peak concentrations of lactic acid and the slower decline in concentrations in defaunated animals suggest that more lactate may have been formed in defaunated animals and/or that it was metabolised more slowly. Although refaunation led to a lowering of lactate concentrations, they remained higher than those in faunated animals, an observation made also by Kayouli *et al.*[19] It appears that defaunation followed by refaunation results in a stable rumen microbial population with a different make-up from that present in the original faunated animal; this is clear from the microbial composition data (Table 2). Whether this effect is linked with changes in fluid dynamics in the rumen,[20] and for how long the resultant microbial population is stable, are subjects that warrant further study.

Chamberlain *et al.*[5] reported that defaunation caused a reduction in the rate of disappearance of lactic acid from the rumen when sheep were given a single intraruminal dose of 50 g D, L-lactic acid. Experiment 2 extended this observation and showed that over a range of increasing dose rates up to 100 g day^{-1}, faunated animals showed higher rates of disappearance of lactate from the rumen (Figure 2). Even at the highest dose level, lactate concentrations in faunated animals had fallen to quite low levels after 3 h whereas corresponding concentrations were not reached in defaunated animals until 7 h after dosing. It should be noted that the impact of the absence of protozoa on the rumen environment would be expected to be more marked than suggested by the results of this experiment since lactate dosing was carried out by way of additions of Na D, L-lactate rather than D, L-lactic acid, the presence of which in the concentrations found in defaunated animals would be expected to be associated with large depressions in ruminal pH (Newbold, Chamberlain and Williams, unpublished).

In the presence of protozoa, ruminal concentrations of total VFA increased as the dose rate of lactate was increased but no such increases occurred in defaunated animals, suggesting a slower rate of metabolism of lactate to VFA in the defaunated animals. In agreement with other studies,[5, 16, 21, 22] the addition of lactate to the faunated rumen caused an increase in the molar proportions of propionate and butyrate. However, in defaunated animals there was no increase in the molar proportion of propionate and although there was a slight increase in the proportion of butyrate when lactate was added, it was not as great as reported previously.[5]

In both experiments defaunation was accompanied by an increase in bacterial numbers, in line with other reports.[19, 23] However, in neither experiment was there any increase in the proportion of lactate-utilising bacteria present. The changes in bacterial numbers in experiment 2 (Table 4) suggest that the increase in lactate-utilising activity in response to intraruminal additions of lactate observed in earlier studies[24, 25] probably derived from a general increase in bacterial numbers rather than from a specific increase in the proportion of lactate-utilisers.

In conclusion, the experiments reported demonstrate the substantial contribution of the protozoa to the ruminal metabolism of lactic acid, irrespective of whether the lactic acid is of endogenous or exogenous origin. The clear suggestion is that the likelihood of digestive disturbances due to an

accumulation of lactic acid in the rumen is diminished by the maintenance of an active protozoal population. In this connection, there is a need for more information on the effects of the chemical composition of concentrate feeds on the protozoal population.

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Cadmium in Roadside Grapes

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The concentration of cadmium in unwashed wine grapes and leaves is considered as a function of the distance from the suspected source, an isolated straight road. Cadmium levels were determined by a.a.s. with electrothermal atomisation in a graphite furnace. The grape and leaf samples display an asymptotic decrease in cadmium concentration (c) according to the distance (d) from the edge of the road. The observed data can be fitted with the exponential function $c=A+B\exp(-Cd)$, the parameters being estimated by least-squares non-linear regression. The simple model allows the determination of the cadmium level at $d\rightarrow\infty$ (A) and at $d=0$ ($A+B$). The results obtained indicate that the effect of the presence of the road is not detectable beyond a distance of 50 m.

Keywords: Roadside pollution; cadmium; wine grapes and leaves; non-linear regression analysis.

1. Introduction

The problem of the lead pollution of roadside crops has been a matter of study for some years,[1–4] but the pollution by other trace metals has been only partially examined. It has been observed that cadmium concentration in grasses and cereals decreases with increasing distance from the road;[5–6] other investigations confirm the earlier findings.[7] In all these researches, however, the sample sizes were too low to allow statistical studies by fitting analytical functions.

The decrease of the lead concentration (c) in crops exposed to automobile exhausts according to the distance (d) from a straight line emitting source has been described by various empirical models, with a view to determining the asymptotic level of concentration which would be exhibited at sites far removed from the source ($d\rightarrow\infty$). A recent paper considered also the polluting effect at $d=0$, suggesting a simple function for the non-linear regression analysis of the plot of c against d for grape and leaf data.[8]

In this paper, the function $c=A+B\exp(-Cd)$ (A, B, $C>0$) is fitted to data on the cadmium pollution of roadside crops, where c is the concentration of cadmium in the polluted material (ng g^{-1}, dry weight) and d is the distance from the edge of the road (m). That the function is suitable can be seen from empirical considerations, viz: (a) at $d=0$ (roadside), $c_0=A+B$ (the intercept on the c-axis); (b) at $d\rightarrow\infty$, $c_\infty=A$ (the asymptote). The data used in testing the function are derived from samples of wine grapes and leaves, which are suitable for the purpose as (a) series of samples are available from a topographical area for which detailed studies have already been done, (b) vineyards often extend right up to the edges of roads and often also surround urban areas, (c) grapes are a direct source of food.

2. Sampling and analytical method

Traminer wine grapes from Angoris (Cormons, Gorizia) were sampled at harvest time. The unwashed bunches were dried at 105°C (weight loss at this temperature is 83.5% as a mean value) and stored in closed flasks in dry atmospheric conditions. Unwashed leaves were sampled at the same time and treated in the same way (weight loss 71.0%).

Charring of 1 g samples was performed at 300°C, followed by ashing at 450°C in a temperature-controlled muffle. The ash was heated with 1 ml of 2 M HNO_3 near to boiling for 1 min; 2 ml of 0.1 M HNO_3 were added. The solution was filtered, the filtrate being collected in a 10 ml calibrated flask; the filter was then washed with 0.1 M HNO_3 until the mark was reached. The resultant solution was appropriately diluted with acid.

The level of cadmium present was determined by atomic absorption spectroscopy (a.a.s.) with electrothermal atomisation in a normal graphite tube, reading absorbance peaks at 228.8 nm. The furnace used was a Perkin Elmer HGA 500 graphite furnace, the following conditions were used: (a) drying at 120°C for 30 s (ramp 10 s, flow 250 ml min^{-1} N_2); (b) charring at 250°C for 30 s (ramp 15 s, flow 200 ml min^{-1} N_2); (c) atomising step 2100°C for 5 s (ramp 1 s, flow 20 ml min^{-1} N_2).

The furnace was fitted with an automatic sampling device mounted on a Perkin Elmer 372 atomic absorption spectrophotometer which was connected to a Hitachi 56 recorder. An 'Intensitron' hollow-cathode lamp was used, with a D_2-background correction.

A two-parameter calibration line was directly used for the determination of the level of cadmium present from the maximum of the absorbance peaks.[9]

The grape samples showed no matrix effect (the calibration line for the pure analyte coincided with that obtained by multiple standard additions), whereas a slight depression of the calibration line was observed with the leaves. In the case of the latter, as the matrix concentration remained unchanged in all solutions, a calibration curve constructed by multiple standard additions of the metal to a typical matrix solution was used for the determination of the amount of cadmium from the absorbance peak.

3. Statistical data processing

The parameters A, B and C of the function $c=A+B\exp(-Cd)$ were estimated by a least-squares non-linear regression program[10–12] run on a CDC Cyber 170-730 D computer. The program also gave estimates of the standard errors of the parameter estimates and the matrix of correlation among them.

Initial estimates of the parameters were derived by visual inspection of the plot of the observations ($A=c_\infty$, $B=c_o-A$) and by trial. The model can be written as a general function $c_i=f(d_i; A, B, C)+e_i$, e_i being the residual ($1\leqslant i\leqslant n$).

Adequacy of fit was determined by examination of the residuals. A graph of e_i as a function of the fitted value c_i provided information on the homogeneity of the variance of the residuals.

The Lin–Mudholkar[13] test was used in order to test the normality of the distribution of the residuals about the regression curve. The test was used in the simple form suggested by Nelson[14] using the r-table given by this author for critical values at a significance level of $\alpha=0.05$. A BASIC program, run on an Olivetti M20ST desk computer, was used for the calculation of r_{obs}.

4. Results and discussion

4.1. Unwashed grapes

Results are reported in Figure 1, the data being ordered according to the distance from the edge of the road (at which $d=0$).

Intralaboratory reproducibility was evaluated from repeated independent analyses of a few samples. In particular, the sample at $d=39.6$ m gave the following values of cadmium content: 20.3, 20.2, 19.3, 22.3, 18.2 ng g^{-1}, dry weight. Hence, $\bar{c}=20.0$, $s^2=2.28$ and the coefficient of variation=7.6%. The analysis of further samples was performed twice, giving a variance of $s^2=28.3$ and a coefficient of variation of 21.5%, estimated from seven duplicated determinations.

Inspection of Figure 1 reveals a monotonic decreasing trend in the plot of the observed levels of cadmium as a function of distance from the roadside, until for $d>50$ m, the level is seen to oscillate around a constant value.

The fitted curve is shown in Figure 1. The parameters of the function are shown in Table 1, together with their estimated variances. When fitting the exponential model in this way, there will

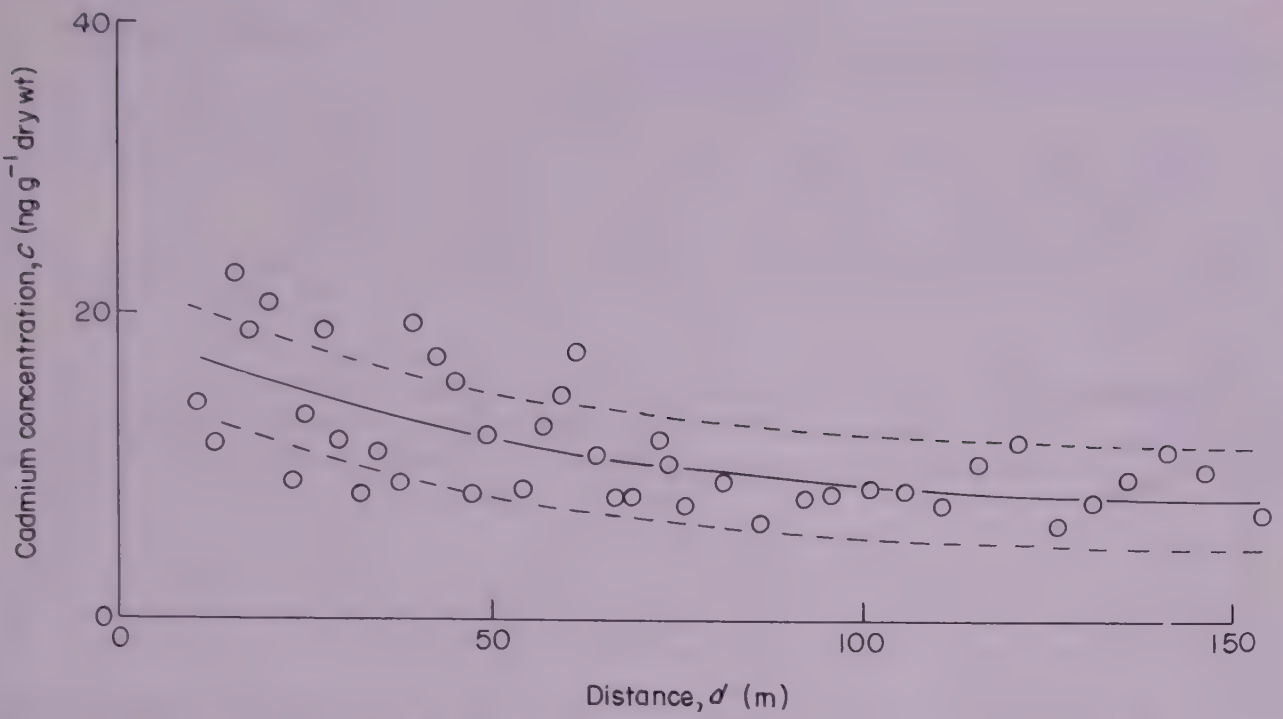

Figure 1. Cadmium concentration (c, ng g^{-1}, dry weight) in unwashed Traminer grapes as a function of the distance (d, m) from the edge of an isolated straight road: (○), observed data; (——), $c=7.906+11.92 \exp(-0.02024d)$; (---), corresponds to $c \pm (s^2_{RES})^{1/2}$.

Table 1. Parameters of the fitted function, $c=A+B \exp(-Cd)$, and their precision for unwashed crops polluted by roadside cadmium

Product	n	A	s^2_A	B	s^2_B	C	s^2_C	$s^2_{RESIDUAL}$
Traminer wine grape at ripening	42	7.906	5.307	11.92	6.387	0.02024	0.0001785	12.42
Traminer leaves	43	43.73	269.3	102.7	307.5	0.01924	0.0001046	620.8

s^2_A, s^2_B, s^2_C are variances of A, B, C; n is the number of data points. $s^2_{RESIDUAL}$, residual variance.

inevitably be a high correlation between A and C. There was no evidence from a Lin–Mudholkar test (at a probability level $\alpha=0.05$), of any deviation from a normal distribution of residuals. Although the variance appears to decrease slightly with increase in distance, the distribution of the residuals about the curve is approximately symmetric, thereby lending support to the use of a least-squares method for the estimation of the parameters, even when the results of a non-parametric run test on the signs of the deviations indicate that the null hypothesis of a random distribution of observations is not acceptable at a significance level of $\alpha=0.05$.

4.2. Unwashed leaves

The leaf samples display a markedly decreasing trend tending to an asymptote, so that data from this source may also be used in testing for the pattern of pollution by cadmium from a linear source. The plot of the observations is shown as Figure 2.

The function $c=A+B \exp(-Cd)$ was fitted to the set of observations; the parameter estimates are given in Table 1. As for the case of the grape samples, the correlation matrix reveals an association between A and C. The analysis of the residuals by means of the Lin–Mudholkar test indicates that they are distributed normally about the fitted curve.

Visual inspection of Figure 2 indicates that the observations are distributed about the curve with an approximately constant standard deviation. Occasionally a small sequence of observations appears to display positive autocorrelation, but the run test on the signs of the deviations indicates no significant difference between the observed deviations and those obtained from a randomly distributed sequence of signs, at least at level of significance of $\alpha=0.05$. The variability indicated by the

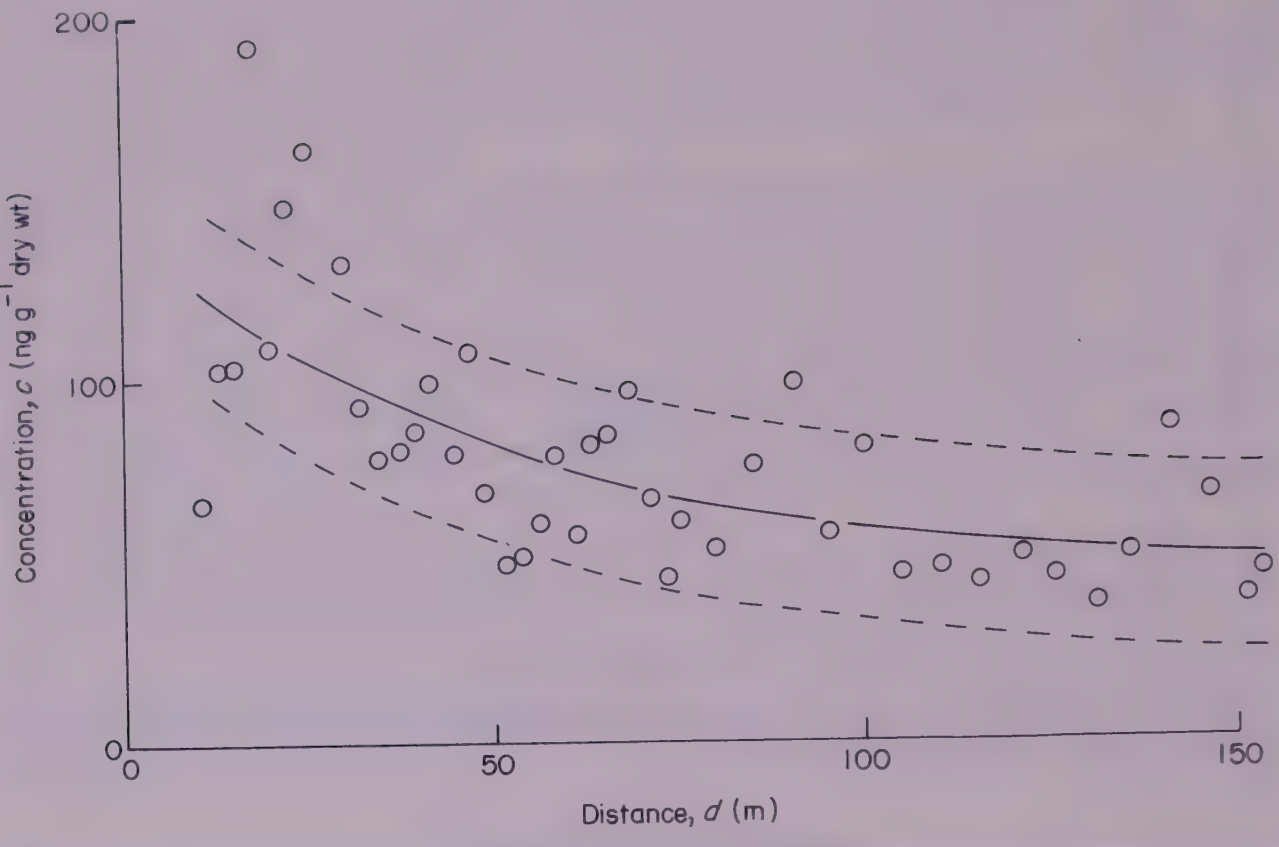

Figure 2. Cadmium concentration (c, ng g^{-1}, dry weight) in unwashed Traminer leaves as a function of the distance (d, m) from the edge of an isolated straight road: (○), observed data; (——), $c=43.73+102.7 \exp(-0.01924d)$. (– – –), corresponds to $c \pm (s^2_{RES})^{1/2}$.

value of $s^2_{RESIDUAL}$ (Table 1) can tentatively be explained by the different exposures of the leaf surfaces to the atmospheric fall-out of cadmium-containing particles and by the effects of washing by rain.

Other factors would probably need to be considered in detail, taking into account that leaf samples (about 0.2 kg each sample) were taken at random on the vine at 1.5 m from ground level. Excluding the stem, each leaf was about 18 cm long. At sampling time, the orientation of leaf surface was not considered. The spatial arrangement of vines, in which the rows are parallel to the line source, may perhaps influence the fall-out of particles near the source, possibly producing the observed oscillating damped trend of the concentration of cadmium. However, no further studies on the atmospheric diffusion near the source have been performed.

The comparison of $C_\infty=A$ for the leaves with that estimated for the grape data, indicates that the former material has an asymptotic cadmium content which is about 5.5 times that of the latter material. For both sets of observations, the effect of the source disappears beyond a distance of 50 m. Leaves at the edge of the road are much more polluted by cadmium, however, than are grapes: for leaves $c_0=A+B=146.4$ whereas $c_0=19.83$ for grapes (ng g^{-1}, dry weight). The ratio $146.4/19.83=7.38$ approaches that obtained from the comparison of surface pollution[15] (leaves 305, grapes 37 cm^2 g^{-1}, dry weight, giving $305/37=8.2$).

Several factors combine to make leaves a suitable material for the study of roadside pollution by cadmium:

(a) the extended period of exposure to the polluted atmosphere; (b) the large surface area compared with grapes; (c) the high degree of roughness of their surfaces compared with those of grape bunches.

Parsley and cabbage have also been used successfully as leafy materials for cadmium research.[16]

4.3. Comparison with lead pollution

The samples considered in this paper were also used for an investigation into lead pollution. The same exponential function was fitted to the observations.

Table 2. Comparison of parameters in roadside pollution of unwashed Traminer grapes (at ripening) and the corresponding leaves by cadmium and lead

	$C_0=A+B$		$C_\infty=A$			
Sample	Cd	Pb	Cd	Pb	C_0(Pb)/C_0(Cd)	C_∞(Pb)/C_∞(Cd)
Grapes	19.83	10977	7.906	441.8	554	55.9
Leaves	146.4	28284	43.73	6264	193	143

A, B are expressed in ng g^{-1}, dry weight.

A decreasing trend in the concentration of lead according to distance from the roadside was observed both in grapes and in leaves.[8] Visual inspection of the data revealed that the observations displayed a decreasing trend, although some evidence of serial correlation was noted in the distribution of the points about the fitted curve.

A comparison of the estimated values of c_∞ shows that the lead content is about 140 times that of cadmium, for the leaf samples; for c_0 ($d=0$) the corresponding ratio in about 190 (Table 2).

As for the case of lead, the short-range effects of pollution from the road disappear beyond a distance of 50 m, thereby lending support to the hypothesis that the particles responsible for the transport of cadmium are the same as those responsible for lead. Various hypotheses have previously been suggested as explanations of the roadside pollution of crops by cadmium,[17] although the discussion has been tentative.

A possible explanation of the common origin of lead and cadmium in atmospheric deposition may be arrived at by considering the transport of both metals by the oil droplets and carbon particles originating in exhaust fumes as these materials have been found to be substantial parts of such emissions.[18]

Further indication of the common origin and transport of roadside cadmium is given in the literature by way of the association of the metal with other trace metals. Cadmium, nickel and zinc, as well as lead, in unwashed parsley and cabbage were found to increase with increasing proximity to a road.[16] Cadmium, chromium, nickel, zinc and lead also increase in rain water close to motorways.[19]

Monitoring cadmium in leafy materials exposed to atmospheric pollution may provide means of detecting an intense source, even when the age of leaded petrol has passed. Lead persists, however, even at large distances from a road, as a consequence of the transport of aerosol matter, whereas cadmium pollution is an environmental concern only for leafy crops in close proximity to the edge of a road.

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Production of Safflower Protein Isolates: Composition, Yield and Protein Quality[a]

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The use of laboratory- and commercially-prepared safflower meal for the production of protein isolates is described. The isolates were obtained by micellisation and isoelectric precipitation techniques, the latter being the most efficient procedure. Previous treatments received by the meal, for example heating, influenced the protein recovery. In general, isolates produced by micellisation were more soluble than samples obtained by isoelectric precipitation. The colour of the protein isolates depended on the isolation technique and type of safflower meal utilised. All isolates had a similar amino acid composition and levels of in-vitro protein digestibility.

Keywords: Safflower; protein isolates; protein solubility; colour; protein quality.

1. Introduction

Safflower (*Carthamus tinctorius* L.) is one of several oilseed crops grown extensively in Mexico, this country being the most important world producer.[1] Safflower plants are relatively drought tolerant, an important consideration for agriculturists in arid or semiarid countries. The oil has a high linoleic acid or oleic acid content and it is widely consumed in many countries. Safflower meal, a by-product of the oil industry, may be separated into two fractions for animal feeding purposes:[2] The high protein fraction contains about 40% protein and 17% fibre and the second (high fibre) fraction contains around 20% protein and 45% fibre.[3]

It has been reported that the high protein fraction is unsuitable for human consumption because of the presence of bitter flavour and cathartic activity when tested on experimental rats.[4] The most widely used process to prepare seed protein isolates involves isoelectric precipitation.[5] After alkaline solubilisation of the proteins (at pH 8–10) and removal of the insoluble material by centrifuging the protein is precipitated by adding acid (to pH 4–6) until the isoelectric point is reached. The only safflower protein isolates reported have been produced using this method.[2, 3] Another process for isolating seed proteins involves precipitation from a neutral salt extract by dilution in cold water.[6, 7] The protein produced in this way has a micelle structure and it seems that hydrophobic interactions play a major role in the stabilisation of such isolates.[7] The aim of the present study was the production, basic characterisation and comparison of protein isolates from safflower meal prepared by two different methods.

2. Experimental

2.1. Safflower samples

Seeds (cv. Gila), harvested in 1984, were gifts of the Government of Sinaloa State. Safflower cake, industrially produced, was obtained from Grasas Mexicanas, S.A., Guadalajara.

[a]Part of this work was presented at the Annual Meeting of The American Association of Cereal Chemists, USA, 1985.

2.1.1. Laboratory-prepared safflower meal (SML)

Seeds were dehulled in a blender with blades covered by rubber tubing, sieved and extracted three times with hexane at 35°C and air-dried. The dehulled seeds were passed through a cyclone mill (UD Cyclone Sample Mill, UD Corp., Boulder, Colorado) using an 80-mesh sieve.

2.1.2. Commerically-prepared safflower meal (SMI)

The commercially-produced cake was passed through a 20-mesh sieve to remove most of the fibre before milling (see 2.1.1).

2.2. Extraction of protein isolates

2.2.1. Micellisation protein (MP)

Samples of both SML and SMI were extracted in a 10% (wt:vol) suspension of sodium chloride of various concentrations (0.1–1.2 M), at pH 5.8 and 7.0, for 30 min (Ultra-Turrax homogeniser, Janke and Kunkel, Staufen, FRG) at 35°C. The extracts were centrifuged at 5000×*g* for 15 min, and the supernatants were diluted with deionised water (1:4). After standing for 30 min at room temperature, the protein was recovered by centrifuging at 10 000×*g* for 20 min and then freeze-dried. All values reported are the average of duplicate experiments.

2.2.2. Isoelectric protein (IP)

This followed reported procedures.[2, 3] A 10% (wt:vol) suspension of SML or SMI was prepared with deionised water and 0.1 M sodium hydroxide to pH 9.0 and homogenised for 30 min. The extracts were centrifuged at 5000×*g* for 15 min, and the supernatants filtered through Whatman No. 4 paper. Protein was precipitated by adjusting to pH 5.5 with 0.1 M hydrochloric acid, separated by centrifuging at 10 000×*g* for 20 min, and then freeze-dried. Results reported are the average of duplicate experiments.

2.3. Proximate composition

Moisture, protein, ether-extractives, crude fibre and ash were determined according to AOAC methods.[8]

2.4. Solubility

Freeze-dried samples (100 mg) were suspended in 10 ml deionised water using 0.1 M hydrochloric acid or 0.1 M sodium hydroxide to obtain a pH range from 2–10.[9] The suspensions were magnetically stirred at 25°C for 30 min, then centrifuged at 10 000*g* for 10 min. Solubility was calculated as percentage of crude protein (N×5.3)[3] present in the supernatant.

2.5. Colour

Colour was determined with a Hunter D25L colorimeter (Hunter Associates Lab, Inc., Reston, VA) and the measurement values expressed as L, a, b, and ΔE colour values.

2.6. Amino acid analysis

The protein samples (20 mg) were hydrolised in a sealed tube containing 10 ml of freshly prepared 6 M hydrochloric acid, under nitrogen at 110°C for 22 h. The amino acids were separated on an automatic analyser (Beckman model 118CL), following the procedure of Spackman *et al.*[10] Amino acid recovery from the hydrolysate was ±90%. Tryptophan was determined using 25 mg of protein samples, hydrolised with papain (EC 3.4.22.2) following the procedure of CIMMYT.[11] Values are means of duplicate runs.

2.7. In-vitro digestibility

In-vitro protein digestibility was determined by the multienzyme technique of Hsu *et al.*[12] Peptidase (EC 3.4.11.1) and trypsin (EC 3.4.21.4) were obtained from Sigma Chemical Co., St.

Louis, Missouri, and α-chymotrypsin (EC 3.4.21.1) from P.L. Biochemicals, Milwaukee, Wisconsin.

3. Results and discussion

Safflower seed had a protein content of 14.6% (Table 1) and an overall composition in agreement with previous studies.[2, 13] The seeds, after dehulling and partial defatting, produced a meal (SML) containing 46.8% protein, whereas the cake from the oil industry (SMI) contained only 28.2% protein. Most protein was extracted from both samples by 0.8 M sodium chloride at pH 7.0 (Figure 1), but much more was extractable from the SML than the SMI samples. All subsequent experiments were carried out under the latter conditions.

Table 1. Chemical composition (as percentage of dry matter) of safflower seed, meals and protein isolates

	Protein (N×5.3)	Ether extractives	Crude fibre	Ash
Seed	14.6	38.8	31.5	2.7
SML	46.8	5.0	7.4	7.2
SMI	28.2	0.9	21.9	8.0
SML-MP	84.8	8.5	0.2	1.3
SML-IP	85.2	6.2	0.1	0.7
SMI-MP	97.4	0.4	0.3	1.0
SMI-IP	85.4	0.6	0.2	0.8

SML=Laboratory-prepared safflower meal; SMI=commercially-prepared safflower meal; MP=micellisation protein isolates; IP=isoelectric protein isolates.

Table 2 shows the amount of protein recovered by the MP and IP processes using both SML and SMI. The protein content of the two MP isolates differed (81.1 and 95.4% for SML and SMI, respectively); whereas the protein content of the IP isolates from both sources were similar at around 80%. The amount of protein recovered was considerably higher for the IP than for the MP procedure (Table 2). Nevertheless, the MP recovery of 44.2% is in agreement with the results of Arntfield *et al.*[7] for fababean isolates.

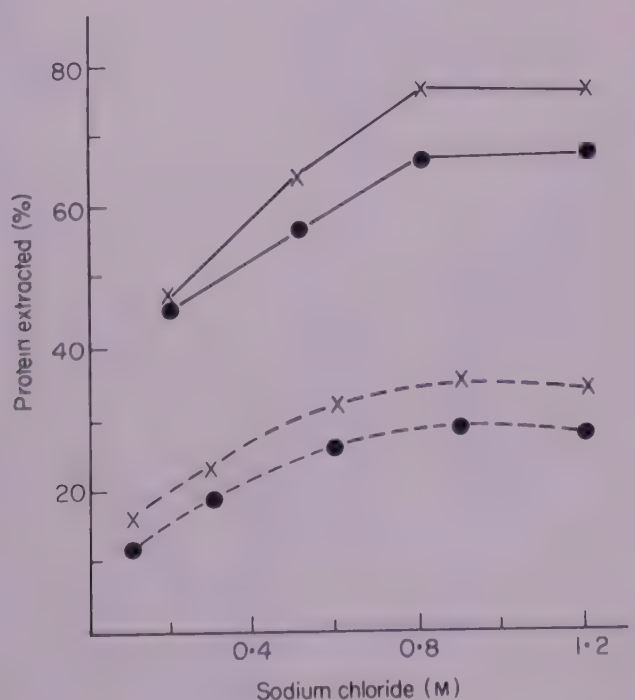

Figure 1. Effect of sodium chloride concentration and pH on the extraction of protein from (——), safflower meal prepared in the laboratory (SML) and (---), a commercial safflower meal (SMI): (•), pH 5.8; (×), pH 7.0.

Table 2. Recovery of protein by micellisation and isoelectric precipitation techniques[a]

	Sample		Protein-isolate			
		Protein content (g)		Protein content		Protein recovered
	Weight (g)	MB	Weight (g)	MC (%)	DM (%)	(percentage of input)
SML-MP	100	44.0	24.0	81.1	84.8	44.2
SML-IP	100	44.0	42.0	82.5	85.2	78.8
SMI-MP	100	26.6	4.8	95.4	97.4	17.2
SMI-IP	100	26.6	15.6	80.0	85.4	46.9

[a]N×5.3.

MC=Moisture content; DM=dry matter; SML=Laboratory-prepared safflower meal; SMI=commercially-prepared safflower meal; MP=micellisation protein isolates; IP=isoelectric protein isolates.

The commercial safflower meal (SMI) extracted poorly (Figure 1); the industrial treatments applied for oil extraction, such as heating, probably affected the solubilisation properties of proteins present in the residual cake. This effect can also be seen in Table 2, were SMI samples had lower protein recoveries compared with the corresponding safflower meals defatted in the laboratory. The detrimental effect on protein extractability on high-temperature treated safflower meal was also noted by Betschart.[3]

The proximate composition of the protein isolates (Table 1) was influenced by the preparative technique. The minimum solubility of all isolates was between pH 5 to 6 (Figure 2). Those isolates produced by the MP technique had a higher solubility than the isolates obtained by the IP procedure, but IP isolates showed a wider range of minimum solubility. It would appear that solubility profiles were more dependent on the isolation technique than on the previous treatment received by the safflower meal (Figure 2).

The SMI preparations were darker than those from SML (Table 3). The MP and IP techniques lowered the L values of SML from 78.9 to 61.2 and 69.9, respectively; which agrees with results from sunflower protein isolates obtained by alkaline extraction.[14] The L reading of SMI-IP

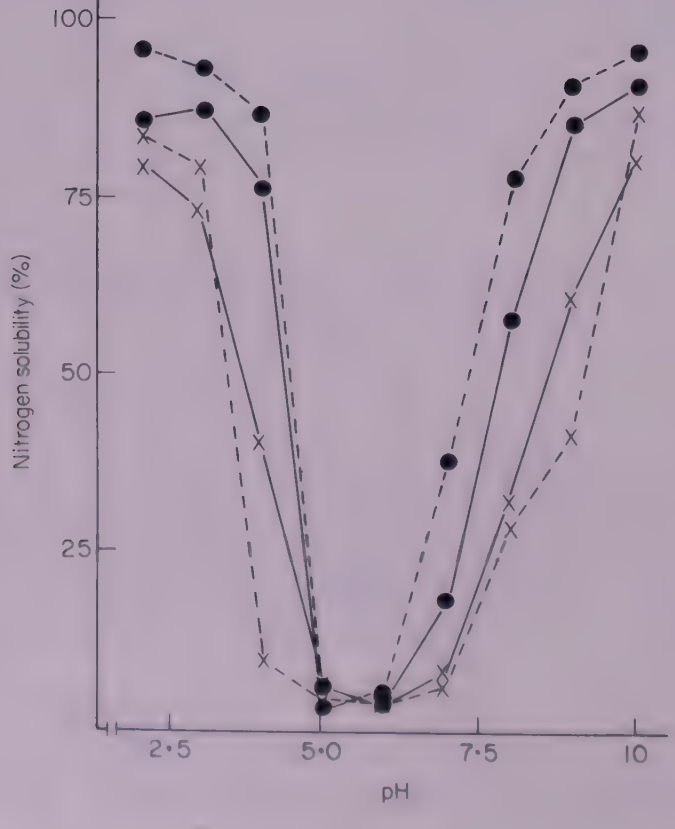

Figure 2. Influence of pH on the solubility of nitrogen from various protein isolates from (——), safflower meal prepared in the laboratory (SML) and (---), a commercial safflower meal (SMI): (•), micellisation protein, (MP) and (×), isoelectric protein (IP) techniques were used.

Table 3. Influence of the isolation procedure on the colour of protein isolates

	L	a	b	ΔE^a
White standard[b]	91.2	−1.0	−1.7	—
SML	78.9	0.02	8.1	15.8
SMI	60.1	1.4	13.5	34.7
SML-MP	61.2	0.03	14.0	33.9
SML-IP	69.9	1.6	14.6	27.0
SMI-MP	70.7	−1.1	12.1	24.7
SMI-IP	51.9	3.1	16.0	43.3

[a] ΔE=Total colour difference=$[(\Delta L)^2+(\Delta a)^2+(\Delta b)^2]^{0.5}$.

[b] Used as reference.

SML=Laboratory-prepared safflower meal; SMI=commercially-prepared safflower meal; MP=micellisation protein isolates; IP=isoelectric protein isolates.

isolates was unexpectedly low at 51.9. The a values did not show a great variation (a=+3.1 to −1.1), indicating the absence of significant red or green coloration in the samples. Both the meals and all of the isolates gave positive b values (b=8.1 to 16.0), indicating some yellow coloration in these products. The low L value of SMI-IP isolate may be due to its yellow pigmentation; which was also found in safflower protein isolates by Latha and Prakash.[13] To summarise, the isolate with the least total colour difference (ΔE) was that obtained by micellisation using samples of SMI.

Table 4 shows that the amino acid composition of the various isolates were similar, although the proportion of nutritionally essential amino acids in the SML isolates was slightly higher than

Table 4. Amino acid composition of safflower protein isolates

	Amino acid content (g 100 g^{-1} protein)			
Amino acid[a, b]	SML-MP	SML-IP	SMI-MP	SMI-IP
Essential				
Lysine	2.51	2.71	2.16	2.30
Threonine	2.56	2.74	1.94	1.89
Valine	3.84	3.47	2.84	3.42
Methionine	0.89	0.85	1.11	0.90
Isoleucine	2.59	2.56	2.24	2.19
Leucine	6.13	6.16	5.78	5.69
Phenylalanine	4.62	4.21	4.63	4.54
Tyrosine	3.00	2.75	3.35	3.21
Tryptophan	1.83	1.83	1.71	1.71
Non-essential				
Histidine	3.02	2.86	3.13	3.65
Arginine	12.15	12.49	11.52	11.66
Aspartic acid	9.21	9.73	9.94	9.90
Serine	4.30	4.25	4.15	4.02
Glutamic acid	22.05	23.12	21.89	21.24
Proline	2.27	2.02	3.62	3.65
Glycine	4.84	5.18	4.82	5.36
Alanine	3.92	2.84	3.74	3.42
Ammonia	2.10	2.06	3.13	3.26

[a] Essential and non-essential for human beings.

[b] Cysteine was not determined.

SML=Laboratory-prepared safflower meal; SMI=commercially-prepared safflower meal; MP=micellisation protein isolates; IP=isoelectric protein isolates.

Table 5. In-vitro digestibility of safflower protein isolates

Protein isolates	Digestibility (%)
SML–MP	89.5±0.3
SML–IP	90.9±0.1
SMI–MP	90.1±0.4
SMI–IP	88.8±0.4
Caseine[a]*	93.8±0.1

[a]Reference protein.

SML=Laboratory-prepared safflower meal; SMI=commercially-prepared safflower meal; MP=micellisation protein isolates; IP=isoelectric protein isolates.

that of preparations made from SMI. The in-vitro digestibility of all the protein isolates was also similar, ranging from 88.8 to 90.9% (Table 5). Neither the amino acid composition nor protein digestibility were greatly affected by the isolation technique used or by different treatments applied to the safflower seed prior to protein extraction.

It is possible that the mild conditions used in the MP procedure allows the protein to retain its native structure; hydrophobic interactions are known to play a major role in the stabilisation of this isolate.[7, 15] The smaller amount of protein recovered by this process, compared to the IP procedure which uses more drastic conditions, might be due to the nature of the protein–protein interactions which appear to exclude low molecular weight components during the micelle formation.[6, 7] The micellisation step needs to be studied in more detail for optimisation.

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Functional Properties of Safflower Protein Isolates: Water Absorption, Whipping and Emulsifying Characteristics[a]

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Safflower meal defatted at the laboratory (SML) and safflower cake provided by an industrial plant (SMI) were used for protein isolation. Isolates were obtained by micellisation (MP) and isoelectric precipitation (IP) techniques. The functional properties studied with these isolates were water and fat absorption, foam expansion and stability, and emulsion activity and stability. Within the experimental conditions of this research, it was observed that the various treatments received previously by the safflower meal, for oil extraction, seemed to have comparatively minor effects on the functional performance of the protein isolates. Only in the case of SML, did the MP isolates have a higher water and fat absorption, and foam expansion than IP samples. For both SML and SMI, the emulsion stability of MP was higher than that of IP.

Keywords: Safflower; protein isolates; functional properties; water absorption; whipping and emulsifying characteristics.

1. Introduction

For plant proteins to be useful and successful in food application, in addition to providing essential amino acids, they should ideally possess several desirable characteristics referred to as functional properties. Water holding capacity is one of the most important functional properties. This capacity is the ability to physically hold water against gravity.[1,2] The fat absorption has been attributed mainly to the physical entrapment of oil.[3]

When an aqueous suspension of protein ingredient is agitated by whipping or by air bubbling, it will entrap air into droplets or bubbles that are surrounded by a liquid film. The interfacial tension between air and water is lowered by the proteins. The good foaming ability of proteins appears to depend on their having a strong affinity for the liquid–air interface and their ability to denature and form cohesive layers possessing sufficient viscosity and mechanical strength to prevent rupture and coalescence of the droplets.[4,5] Moreover, proteins constitute an important group of emulsifiers because as indicated they cause a reduction of interfacial tension, they form rigid interfacial films, and they possess charged groups.[5,6,7]

Numerous studies have been published on the functional properties of plant proteins[2–4,8–11] including safflower proteins.[12,13] In these studies, protein isolates or concentrates were usually obtained by the isoelectric precipitation technique; the isoelectric point of safflower protein is about 5.0 or slightly higher.[14] In another process developed for isolating plant proteins, the protein is precipitated from a neutral salt extract by dilution in cold water.[15] The protein produced in this way has a micelle structure and it seems that hydrophobic interactions play a major role in the stabilisation of such an isolate.[16] The present research was done to compare the functional properties of safflower protein isolates, obtained by these two techniques; two different types of safflower meals were used, as described previously.[17]

[a]Part of this work was presented at the Annual Meeting of the American Association of Cereal Chemists, USA, 1985.

2. Experimental

2.1. Protein isolates

Protein isolates were obtained by a micellisation technique and here are referred to as micellisation protein (MP). When protein isolates were obtained by isoelectric precipitation, they were termed isoelectric protein (IP). Both MP and IP were produced from safflower meal defatted at the laboratory (SML) under gentle and controlled conditions,[17] and from safflower meal provided by an industrial plant (SMI); SML was defatted with hexane at 35°C, whereas SMI was mechanically pressed in the expeller reaching rather high temperatures (90–93°C) and then extracted with hexane at 35°C.

The MP isolates were obtained from SML and SMI samples homogenised in a 10% (wt:vol) suspension of 0.8 M sodium chloride at pH 7.0. The extracts were centrifuged and the supernatants were diluted with water (1:4). After standing for 30 min, the protein was recovered by centrifuging and then freeze dried.[17] The IP isolates were produced in 10% (wt:vol) suspensions of SML or SMI samples at pH 9.0. After centrifuging, the protein in the supernatants was precipitated at pH 5.5. The recovered protein was then freeze dried.[14]

2.2. Water and fat absorption

Water absorption was measured following the methods described by other workers.[10,11] Protein samples (0.5 g) and deionised water (5 ml) were stirred for 1 min in a graduated tube which was allowed to stand for 30 min, at about 25°C, then centrifuged at 1600×*g* for 25 min. The volume of free water was measured and the retained water was expressed as ml of water absorbed per g of protein.

Fat absorption was determined according to Lin *et al.*[18] except that olive oil was used here.

2.3. Whipping properties

Foam expansion and foam stability were taken as indices of whipping properties. These indices were assessed using the procedure described by Kabirullah and Wills[10] and Canella.[8] This involved blending 50 ml of a protein suspension (1%, adjusted to the required pH level) in an Ultra-Turrax homogeniser (Janke and Kunkel, Staufen, FRG), at 12 000 rev min^{-1} for 1 min at about 25°C and determining the volume of foam (in ml) that was present above the surface of the liquid contained in a glass cylinder of 100 ml. Foam expansion was expressed as follows:

$$\text{Foam expansion (\%)} = \frac{\text{Vol after whipping} - \text{Vol before whipping}}{\text{Vol before whipping}} \times 100$$

After standing for 30 min, the volume of foam (in ml) remaining was recorded as foam stability and it was calculated as follows:

$$\text{Foam stability (\%)} = \frac{\text{Vol after standing} - \text{initial Vol}}{\text{Vol after whipping} - \text{initial Vol}} \times 100.$$

2.4. Emulsifying properties

Emulsifying activity and emulsion stability were determined at various pH and at ~25°C by the method of Wang and Kinsella.[11] Calculations were as follows:

$$\text{Emulsion activity (\%)} = \frac{\text{Vol of emulsified layer}}{\text{Total vol}} \times 100.$$

$$\text{Emulsion stability (\%)} = \frac{\text{Vol of emulsified layer remaining}}{\text{Vol of initial layer}} \times 100.$$

3. Results and discussion

The water holding capacity of MP isolates (Table 1) is of about the same level as that reported for oat protein isolates,[19] but quite low compared to sunflower isolate.[10] It is expected that increasing the pH and adjusting the absorption values for solubilised protein will enhance this capacity, as observed by Hutton and Campbell.[3]

It was observed that the isolation techniques affected the water absorption capacity of the protein samples in a different way. MP isolates from SML and SMI had higher water absorption values than those obtained by the isoelectric precipitation technique (IP) (Table 1). The MP isolates had a higher water absorption capacity than IP samples probably because more polar and ionic groups were exposed, allowing greater interaction with the surrounding water by means of hydrogen bonding. Other factors that affect the protein–water interaction include the physical structure (for example, particle and pore size) and the chemical properties (for example hydratability) of the protein, as well as the protein conformation and environmental factors; conformational changes in the protein molecules may expose previously buried amino acid side chains, thereby making them available to interact with water.[3]

According to Kinsella,[4] fat absorption of food products is an important functional property because it improves mouthfeel and flavour retention. Fat absorption of all isolates (Table 1) is favourably compared to that of other isolates reported in the literature.[3,11,18,20]

For SML samples at the various pH values tested, MP isolates showed a better foaming ability than the proteins obtained by isoelectric precipitation (Table 2). This trend was not observed with the SMI-MP and SMI-IP isolates, which showed a high increase in foam expansion at pH 2 as compared to the values at pH 4 to 10. Table 3 shows that MP isolates from SML and SMI presented the best stability at pH 4, whereas the highest stabilities for IPs were found at the pH extremes. It is difficult to compare these data with other sources in view of the variety of methods used. The solubility profile of all protein isolates[17] showed minimum values between pH

Table 1. Water and fat absorption of safflower protein isolates[a]

	Water absorption (ml g^{-1} protein)	Fat absorption (ml g^{-1} protein)
SML-MP	1.80 ± 0.13^{a}	2.82 ± 0.00^{a}
SML-IP	1.29 ± 0.12^{c}	2.44 ± 0.24^{b}
SMI-MP	1.58 ± 0.12^{b}	2.47 ± 0.00^{b}
SMI-IP	$1.41\pm0.00^{b,c}$	2.27 ± 0.14^{b}

[a]Value in columns with different letter are significantly different ($P\leq0.05$) by Duncan's Multiple Range Test.

SML=Safflower meal prepared at the laboratory; SMI=safflower meal from industry; MP=micellisation protein isolates; IP=isoelectric protein isolates.

Table 2. Effect of pH on the foam expansion of safflower protein isolates (%)[a]

	pH				
	2	4	6	8	10
SML-MP	54.7 ± 1.2^{b}	25.3 ± 2.3^{cd}	22.7 ± 1.5^{de}	20.0 ± 0.0^{ef}	22.0 ± 2.0^{de}
SML-IP	52.7 ± 3.0^{b}	16.7 ± 1.1^{g}	20.0 ± 0.0^{ef}	16.7 ± 1.1^{g}	18.7 ± 2.3^{fg}
SMI-MP	69.3 ± 3.0^{a}	28.0 ± 2.0^{c}	12.0 ± 0.01^{i}	25.3 ± 1.1^{cd}	23.3 ± 3.0^{de}
SMI-IP	54.7 ± 2.3^{b}	54.7 ± 1.1^{b}	16.0 ± 0.0^{gh}	13.3 ± 1.1^{hi}	22.0 ± 3.5^{de}

[a]Values in columns with different letter are significantly different ($P\leq0.05$) by Duncan's Multiple Range Test.

SML=Safflower meal prepared at the laboratory; SMI=safflower meal from industry; MP=micellisation protein isolates; IP=isoelectric protein isolates.

5 to 6. Investigators working on foaming properties believe that foam expansion and stability are at a maximun near the pI of a protein.[6] However, based on the present results it seems that the effect of pH on the properties of these particular isolates is extremely complex.

Table 4 shows slight variations in emulsion activity of all samples as a result of pH changes, except SML-IP at pH 4 and 6. It can be seen that emulsion activity of all isolates was not much influenced by the different treatments applied to the raw material and by the isolation technique used. In other words, there were not big differences in emulsion activity between the sample defatted at the laboratory under gentle conditions, and the safflower cake from an industrial extraction plant; this was also the case for the two isolation procedures used. It was noted that the emulsion stability was higher for the isolates obtained by the micellisation procedure as compared to that of the IP isolates (Table 5); pH variations produced about the same slight changes in emulsion stability as those exhibited by emulsion activity except, again, at pH 4 and 6 for SML-IP. It has been found that soya bean protein-stabilised emulsions, upon heating, were quite unstable in the pH range around the pI.[5] In this study SML-MP, SMI-MP and SMI-IP

Table 3. Effect of pH on the foam stability of safflower protein isolates (%)[a]

	pH				
	2	4	6	8	10
SML-MP	27.8 ± 2.6^{fg}	86.9 ± 4.3^{ab}	81.9 ± 1.2^{b}	53.3 ± 5.7^{de}	11.9 ± 4.1^{h}
SML-IP	64.8 ± 0.8^{c}	27.8 ± 4.8^{fg}	33.3 ± 5.8^{f}	51.9 ± 3.2^{de}	55.2 ± 5.0^{d}
SMI-MP	51.8 ± 5.5^{de}	90.6 ± 3.4^{a}	27.8 ± 9.6^{fg}	21.2 ± 5.2^{g}	68.6 ± 1.7^{c}
SMI-IP	66.0 ± 3.0^{c}	8.9 ± 3.1^{h}	45.8 ± 7.2^{e}	54.8 ± 4.1^{de}	55.4 ± 8.0^{d}

[a]Values in columns with different letter are significantly different ($P\leq0.05$) by Duncan's Multiple Range Test.

SML=Safflower meal prepared at the laboratory; SMI=safflower meal from industry; MP=micellisation protein isolates; IP=isoelectric protein isolates.

Table 4. Effect of pH on the emulsion activity of safflower protein isolates (%)[a]

	pH				
	2	4	6	8	10
SML-MP	52.5 ± 0.3^{bcde}	53.7 ± 0.3^{bc}	53.0 ± 0.6^{bcde}	51.4 ± 0.8^{def}	56.4 ± 0.7^{a}
SML-IP	56.6 ± 0.3^{a}	45.1 ± 0.7^{h}	37.6 ± 4.0^{i}	57.5 ± 1.6^{a}	53.8 ± 0.8^{bc}
SMI-MP	54.3 ± 2.3^{a}	49.0 ± 0.0^{g}	49.1 ± 1.0^{fg}	52.0 ± 0.7^{cde}	53.4 ± 0.7^{bcd}
SMI-IP	53.9 ± 0.7^{bc}	49.7 ± 2.1^{fg}	51.4 ± 0.5^{def}	50.9 ± 0.3^{efg}	53.2 ± 0.3^{bcd}

[a]Values in columns with different letter are significantly different ($P\leq0.05$) by Duncan's Multiple Range Test.

SML=Safflower meal prepared at the laboratory; SMI=safflower meal from industry; MP=micellisation protein isolates; IP=isoelectric protein isolates.

Table 5. Effect of pH on the emulsion stability of safflower protein isolates (%)[a]

	pH				
	2	4	6	8	10
SML-MP	97.5 ± 1.0^{a}	91.0 ± 2.9^{de}	92.2 ± 1.5^{cd}	95.0 ± 1.1^{abc}	94.2 ± 0.8^{bc}
SML-IP	88.3 ± 1.8^{e}	8.5 ± 2.0^{h}	2.8 ± 0.3^{i}	74.9 ± 3.2^{f}	57.0 ± 2.8^{g}
SMI-MP	92.2 ± 1.4^{cd}	94.9 ± 1.1^{abc}	97.4 ± 1.0^{a}	97.1 ± 1.1^{a}	96.3 ± 1.5^{ab}
SMI-IP	88.7 ± 1.4^{e}	93.9 ± 2.7^{bc}	90.6 ± 1.9^{de}	92.5 ± 2.0^{cd}	92.9 ± 1.0^{cd}

[a]Values in columns with different letter are significantly different ($P\leq0.05$) by Duncan's Multiple Range Test.

SML=Safflower meal prepared at the laboratory; SMI=safflower meal from industry; MP=micellisation protein isolates; IP=isoelectric protein isolates.

emulsions (Table 5) may have exhibited within the isoelectric pH range an appropriate balance between attractive and repulsive forces, thereby preventing coalescence of droplets; this balance may have counteracted the aggregation of proteins, giving as a result emulsions of remarkable stabilities. This interesting phenomenon requires more detailed studies.

In conclusion, although it might be that a dramatic reduction in the functional properties of the SMI proteins would occur as a result of the harsh heat treatments applied during oil extraction this was not generally the case with the safflower protein isolates. Only in a few specific cases did the previous treatments received by the safflower meals[17] appear to be important. In relation to the isolation technique, for SML samples MP exhibited a higher water and fat absorption, and foam expansion than IP; in emulsion stability, for a given safflower meal, MP performed better than IP. Further work is in progress at the molecular level to explain these functional performances. Research work is also needed to compare the functional properties of MP and IP samples with established functional protein isolates.

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Free α-amino Nitrogen Production in Sorghum Beer Mashing

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Free α-amino nitrogen (FAN) is an essential nutrient for yeast growth during fermentation. Under normal conditions of sorghum beer mashing, 60°C at pH 4.0, production of FAN by proteolysis accounts for approximately 30% of wort FAN, the remaining 70% being preformed in the malt and adjunct. The quality of the FAN in sorghum beer worts is good as it does not contain a high percentage of proline. Optimum conditions for FAN production during mashing are 51°C and pH 4.6. Wort FAN was increased proportionally by raising the ratio of sorghum malt to adjunct and conversely decreased by raising the ratio of adjunct to malt. FAN was also increased by the addition to the mash of a microbial proteolytic enzyme. Wort FAN is directly proportional to malt FAN.

Keywords: Sorghum beer; mashing; free α-amino nitrogen.

1. Introduction

Mashing is the process in brewing where the enzymes developed during the malting of grain are used to degrade starch, protein and other reserves in the malt and adjunct into a fermentable extract. Free α-amino nitrogen (amino acids and peptides), the product of protein degradation is required as a nutrient for yeast growth,[1] being synthesised into the structural and enzyme proteins of the yeast.

In Africa, the traditional alcoholic beverage is sorghum beer which differs from European-type beer in that it is brewed with malted sorghum and not barley malt.[2] The beer is characterised by being opaque due to suspended solids and by being flavoured by lactic acid rather than hops.[2] In the brewing of sorghum beer it is usual to use a high ratio of cereal adjunct to malt,[3] normally maize grits or sorghum grain.[2] Cereal adjuncts are known to contribute very little to the final free α-amino nitrogen (FAN) content of the wort.[4] Thus, a consequence of brewing with a high ratio of adjunct to malt is that the FAN content of the wort may be too low to support good yeast growth.[5, 6] Limited yeast growth will in turn result in incomplete fermentation of sugars and hence a beer with below the desired level of alcohol.[7] It is, therefore, essential that the brewing process should result in a wort containing sufficient FAN. To ensure this, knowledge is required concerning the production of FAN during mashing.[6, 8] This paper describes research carried out to provide such knowledge for brewing sorghum beer.

2. Experimental

2.1. Materials

A number of different sorghum malts were used in this study. Six malts were prepared in the laboratory from cultivar NK283 with a wide range of FAN contents. In addition, 40 malts from industrial maltsters of cultivars unknown, were obtained from sorghum beer breweries. Milezyme AFP, a microbial proteinase, was kindly donated by Seravac, Fine Chemicals Corporation (Pty) Ltd.

2.2. Mashing

Mashing was carried out in a BRF mashing bath (Crisp Malting Limited, Great Ryburgh, England).

The mashing procedure was based on the Reef-type process where an infusion mash at 60°C of up to 2 h at pH 4.0 is used.[2]

Adjunct consisting of maize grits (32.37 g dry wt) and sorghum malt (3.6 g dry wt) was cooked at 100 kPa for 10 min in 280 ml distilled water plus the quantity of 1 M lactic acid required to bring the final mash pH to 4.0. After cooking, the mash was cooled to 60°C and made up to 330 g minus the weight of conversion malt to be added, with distilled water. Mashing was normally carried out using 9.6 g dry wt of sorghum malt for conversion. In various experiments, the effect of altering mash pH, temperature, the proportion of conversion malt to maize grits and the addition of a microbial proteinase, were investigated.

2.3. Analyses

The FAN content of sorghum malt was determined by extraction of 1 g malt with 40 ml 5% (50 g litre^{-1}) trichloroacetic acid at 30°C for 1 h. After centrifuging at 4500×g for 10 min, 1 ml of clear supernatant was diluted to 25 ml with distilled water and FAN measured using the EBC ninhydrin method.[9]

To determine the FAN content of mashes during conversion, protease activity was stopped by mixing 10 g samples with an equal weight of 10% (100 g litre^{-1}) trichloroacetic acid. After centrifuging to obtain a clear supernatant, FAN was measured using the EBC ninhydrin assay.[9] The supernatant was also used to determine the total non-protein nitrogen content of the mashes. Nitrogen was measured by an automated Berthelot alkali–phenate method,[10] after digestion of the samples with conc. sulphuric acid containing 20% K_2SO_4 (200 g litre^{-1}) and 0.1% SeO_2 (1 g litre^{-1}). The amino acid composition of the total non-protein nitrogen was determined. The trichloroacetic acid was removed from the supernatants by extraction with diethyl ether and after freeze-drying, the material was hydrolysed into amino acids with 6 M hydrochloric acid at 110°C for 22 h under vacuum. Analysis was made using an LKB Alpha amino acid analyser.

The effect of conversion on the nitrogenous composition of the mash was determined. Mashing was carried out at 55 and 60°C, pH 4.0. Immediately after addition of the conversion malt to the adjunct and after 2 h conversion, the contents of individual mashing beakers were frozen with an acetone–dry ice mixture, then freeze-dried. The milled freeze-dried material was then extracted using an Osborne-type protein fractionation procedure[11] to determine the quantity of low molecular weight nitrogen (LMWN), albumin plus globulin proteins and prolamin plus glutelin proteins. Protein was measured as nitrogen, as described above.

3. Results and discussion

Table 1 shows the effect of mashing on the nitrogenous composition of the mash. The results are the mean of duplicate mashes. At the start of conversion 84% of the nitrogen was in the form of prolamin and glutelin proteins and only 8–9% was LMWN. As a result of mashing at both 55 and 60°C, there was an increase in LMWN. This nitrogenous fraction is defined in terms of being small enough to pass through a dialysis membrane. It can, therefore, be considered as more or less synonymous with FAN. Parallel with the increase in LMWN, there was a decrease in prolamins and glutelins. In contrast, the albumin and globulin proteins showed a slight increase. These results show that during sorghum beer mashing, some of the prolamin and glutelin proteins are degraded by the malt proteolytic enzymes into FAN.

In the sorghum grain, prolamins and glutelins function as storage proteins and are degraded during germination.[11] It, therefore, appears that the mechanism of protein degradation in sorghum beer mashing is the same as that which occurs during sorghum germination. However, the magnitude of the increase in LMWN during mashing was relatively small, so that after 2 h this fraction still only made up 10.6–12.4% of the total nitrogen. This finding is in agreement with data from European beer brewing where amino acids in the wort correspond to about 12% of the total nitrogen in the barley.[5]

Progress curves for the release of FAN and total non-protein nitrogen during mashing are shown in Figure 1. In both cases the percentage increase was some 30–35% as was also shown in Table 1.

Table 1. Effect of mashing on the nitrogenous composition of the mash[a] (mg N kg^{-1} mash)

	Mashing temperature 55°C			Mashing temperature 60°C		
	Mashing time of 0 min	Mashing time of 120 min	Change	Mashing time of 0 min	Mashing time of 120 min	Change
Low molecular weight nitrogen	163.7 (8.9)[b]	230.2 (12.4)	+66.5 (+3.5)	149.4 (8.2)	190.9 (10.6)	+41.5 (+2.4)
Albumins+globulins	125.8 (6.8)	138.2 (7.4)	+12.4 (+0.6)	141.6 (7.7)	154.0 (8.5)	+12.4 (+0.8)
Prolamins+glutelins	1554.3 (84.3)	1488.5 (80.2)	−65.8 (−4.1)	1536.1 (84.1)	1456.7 (80.9)	−79.4 (−3.2)
Total	1843.8	1856.9		1827.1	1801.6	

[a] Mashes carried out using an NK283 malt, FAN content 102 mg 100 g^{-1}.

[b] Figures in parentheses are the relative proportions of the nitrogenous fractions.

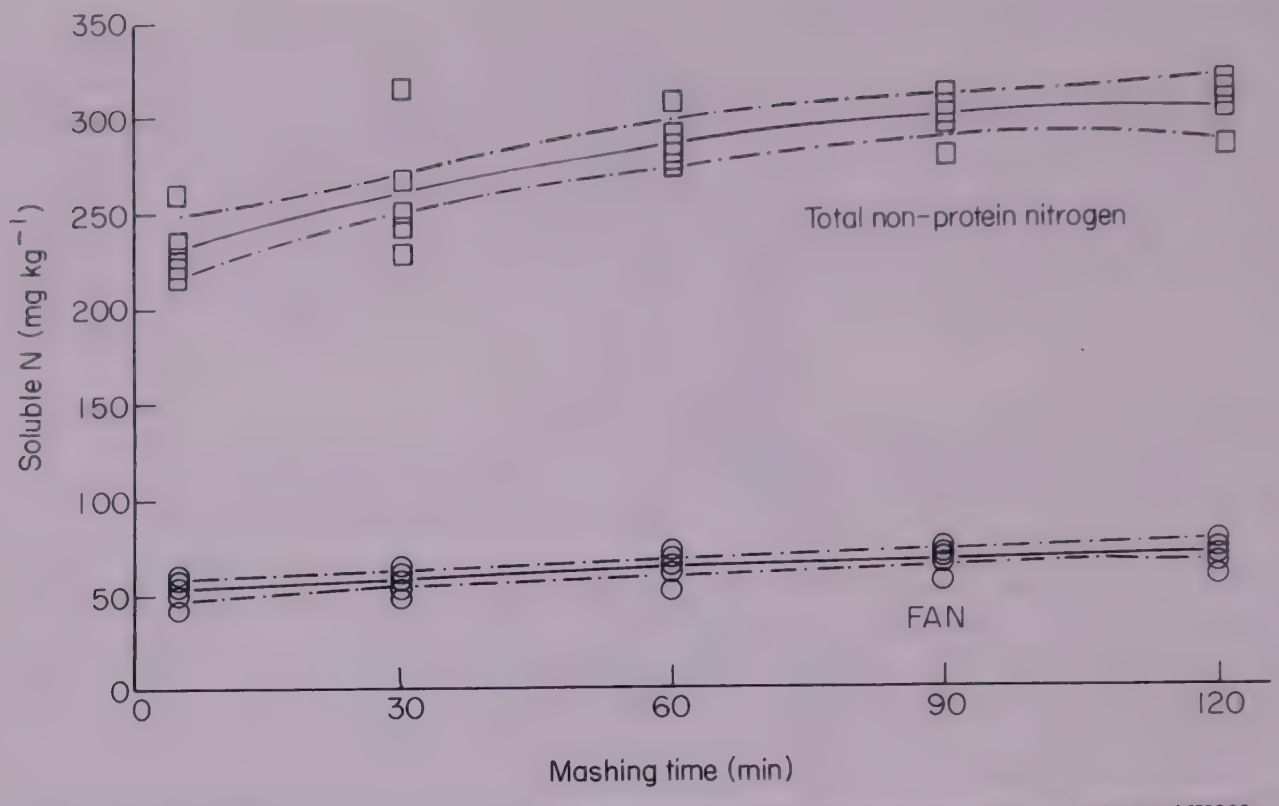

Figure 1. Effect of mashing on FAN and total non-protein nitrogen production: mashing carried out using an NK283 malt. FAN content 102 mg 100 g^{-1}.

This means that under normal mashing conditions approximately 30% of the FAN in the wort resulted from proteolysis during mashing and 70% was from preformed FAN in the malt and adjunct. In European beer brewing there is some controversy as to the relative importance of the malt and mashing to the provision of FAN in the wort. It has been suggested that FAN release during high temperature (65°C) infusion mashes is minimal.[8] However, the data in Table 1 and Figure 1 show that this is not the case with the 60°C infusion mashing procedure employed in sorghum beer brewing. Figure 1 also shows that the ratio of total non-protein nitrogen to FAN was just over 4 to 1. This indicates that the α-amino nitrogen in the mash consisted of a considerable proportion of small peptides in addition to free amino acids. The fact that the ratio remained more or less constant during mashing suggests that both proteinase and peptidase enzymes were active. An increase in just total non-protein nitrogen would have implied that only proteinases were active, whereas an increase in just FAN would have suggested that only peptidases were active.

The amino acid composition of the wort FAN is important because it affects its yeast-feeding value. Jones and Pierce[12] classified amino acids into four groups on the basis of their rate of uptake by yeast during fermentation. Group A amino acids, e.g. glutamic acid, are almost immediately absorbed from the wort. Group B, e.g. valine, are not removed rapidly but are absorbed gradually during fermentation. Group C, e.g. glycine, are only absorbed after the removal of group A amino acids. Group D, the imino acid, proline, is only slightly absorbed from wort under the anaerobic conditions of fermentation. Table 2 shows the amino acid composition of the total non-protein nitrogen after 5 and 120 min mashing at 60°C, pH 4.0 using sorghum malts of two different FAN levels. It can be seen that mashing resulted in very little change in the amino acid composition. With both the high and low FAN malts, group A amino acids made up approximately 50% of the total. Aspartic acid and glutamic acid were the most abundant. The group D imino acid proline accounted for only 9.0 and 10.9% of the total. These results are in agreement with those from a previous investigation into the composition of sorghum malt FAN where it was found that aspartic and glutamic acid were the most abundant amino acids and proline was a relatively minor component.[11] This shows that the sorghum malt is mainly responsible for the non-protein nitrogen composition of sorghum beer mashes. The non-protein nitrogen composition of these mashes, however, differed

Table 2. Effect of mashing on the amino acid composition of the total non-protein nitrogen (g amino acid 100 g^{-1} amino acids analysed)

	FAN content of malts[a]			
	228 mg FAN 100 g^{-1}		102 mg FAN 100 g^{-1}	
Amino acid	Mashing time of 5 min	Mashing time of 120 min	Mashing time of 5 min	Mashing time of 120 min
Group A				
Aspartic acid	21.2	18.5	17.9	16.1
Threonine	2.9	3.4	3.2	3.4
Serine	3.5	3.6	3.7	3.8
Glutamic acid	19.1	18.9	17.3	16.8
Lysine	2.6	3.3	3.5	4.1
Arginine	2.3	2.8	2.5	3.6
Sub-total	51.6	50.5	48.1	47.8
Group B				
Valine	4.8	4.8	4.0	4.5
Methionine	1.0	1.3	1.1	1.3
Isoleucine	2.8	3.1	2.4	2.8
Leucine	5.3	6.4	4.5	5.7
Histidine	4.1	4.3	4.3	4.2
Sub-total	18.0	19.9	16.3	18.5
Group C				
Glycine	3.6	3.7	4.4	4.5
Alanine	7.0	7.0	6.6	6.8
Tyrosine	4.2	4.2	3.8	4.6
Phenylalanine	2.4	2.0	2.2	1.8
Ammonia	4.2	3.4	4.9	4.9
Sub-total	21.4	20.3	21.9	22.6
Group D				
Proline	8.8	9.0	13.6	10.9

[a] Mashing carried out using malts of cultivar NK283.

considerably from those using barley malt where proline is the predominant free amino acid and can account for up to one-third of the total.[12] The much lower proline content of sorghum beer mashes suggests that the FAN has a better yeast-feeding value than that of barley malt mashes. The probable reason for the difference in composition is that sorghum prolamin from which the non-protein nitrogen is derived, contains a much lower percentage of proline than barley hordein, 9.9 as against 22.4 to 23.1.[13]

As brewing with high ratios of adjunct to malt may result in wort FAN being limiting,[5, 6] methods of influencing FAN production during mashing were investigated. To determine the effect of the malt, mashes were carried out using 40 malts from industrial maltsters. Figure 2 shows the relationship between wort FAN and malt FAN. It can be seen that wort FAN was highly significantly correlated with malt FAN. Thus, the use of a high FAN malt will result in a high FAN wort and conversely a low FAN malt will produce a low FAN wort. There was, however, no significant correlation between malt FAN and FAN production during mashing, $r = -0.153$.

Jones[14] described five ways of varying the FAN in wort, among these were alteration of the composition of the mash and altering the method of mashing. Altering the composition of the mash was described as the simplest practical method. Figure 3 shows the effect on the FAN at the end of 2 h mashing of using different ratios of conversion malt to maize grits, whilst keeping the total solids input constant. It can be seen that the amount of FAN was directly related to the proportion of conversion malt in the mash. Increasing the proportion of malt resulted in higher FAN and decreasing the proportion resulted in low FAN. With all but the lowest FAN malt there appeared to be a slight exponential increase in FAN with malt content. A possible explanation is that with increasing malt there was an increasing proportion of proteolytic enzymes in the mash, resulting in

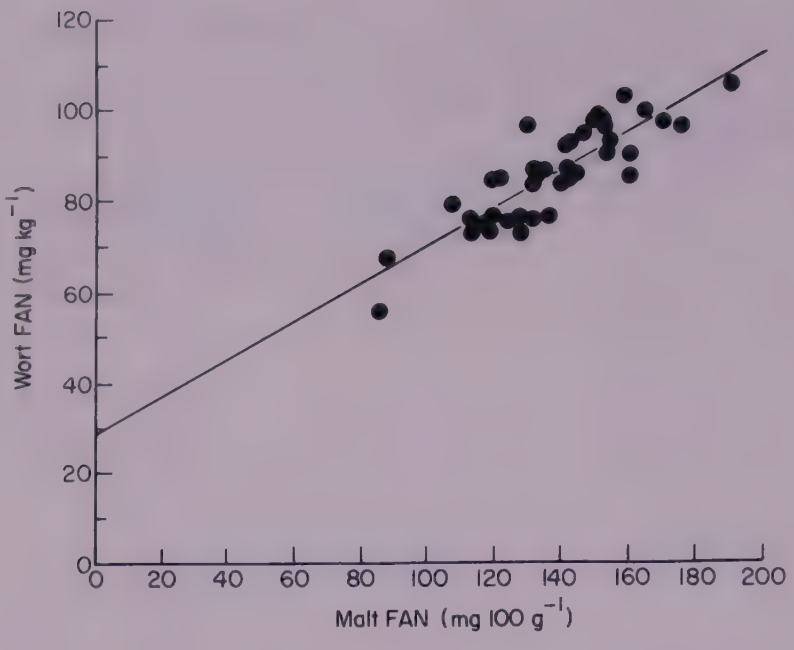

Figure 2. Relationship between wort FAN and malt FAN. Mashing carried out using 40 malts from industrial maltsters: $y=0.42x+28.42$; $r=0.861$.

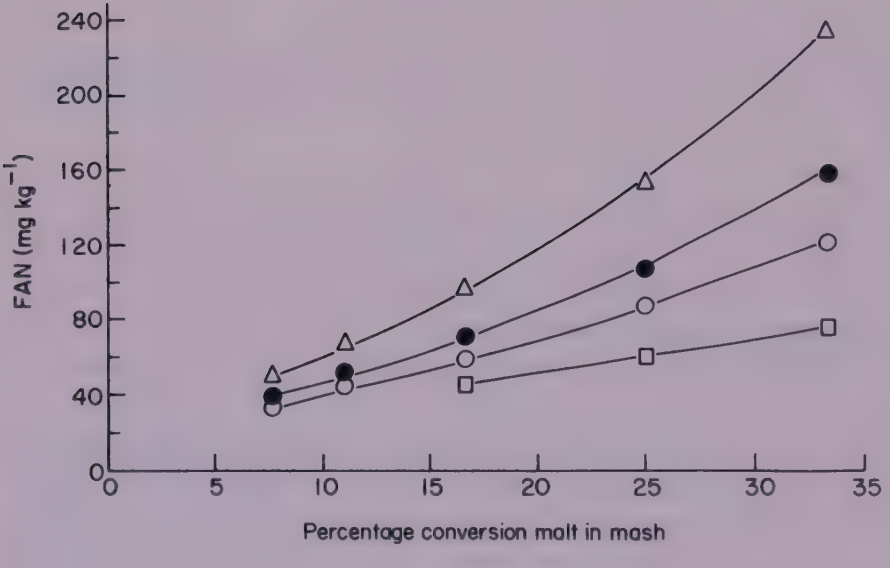

Figure 3. Effect on FAN of using different amounts of conversion malt in the mash. Mashing carried out using NK283 malts. Malt FAN (mg 100 g^{-1}): (□), 94; (○), 143; (●), 215; (△), 339.

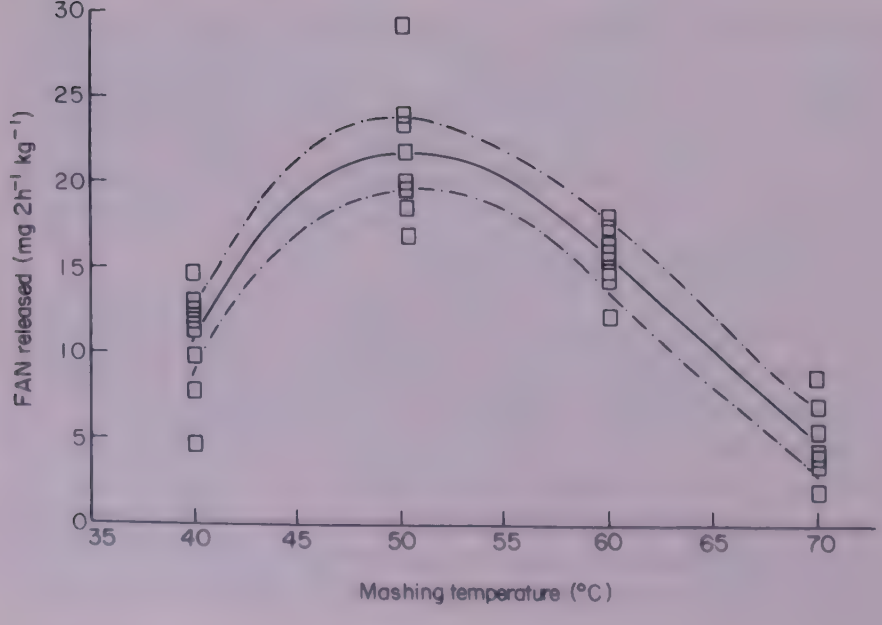

Figure 4. Effect of mashing temperature on FAN production. Mashing carried out using an NK283 malt, FAN content 102 mg 100 g^{-1}: (—·—), 95% confidence limits.

higher FAN production during mashing. When brewing with barley as adjunct in barley malt mashes, it has been found that wort α-amino nitrogen was proportional to the amount of malt, throughout the range 0 to 100% barley.[5]

With regard to altering the method of mashing, Figure 4 shows the effect of mashing temperature on FAN production. The optimum temperature was around 51°C and significantly less FAN was released at 40 and 60°C. The latter temperature is normally used in this brewing process as it is optimum for sugar production.[3] A few studies have been carried out on the proteolytic enzymes of germinating sorghum, the enzymes responsible for FAN release during mashing.[15–17] Interestingly, a temperature optimum of 50°C was found for a purified protease enzyme.[15] In lager brewing a period of mashing is usually carried out at the optimum temperature for protease activity in order to increase FAN production. This period or 'protein rest' is in the temperature range 45–55°C[5, 8, 18] which is probably optimum for the heat-labile proteinase enzymes,[5] the rate-limiting enzymes of FAN release during mashing.[6]

In the brewing procedure under study, mashing is carried out at pH 4.0, rather than the natural pH of the mash. The low pH is attained by souring some of the malt in the grist by *lactobacillus* fermentation.[2] Mashing at low pH has the effect of preventing total starch degradation so that the beer retains its characteristic body and viscosity.[3] Figure 5 shows the effect of pH on FAN production during mashing. The optimum pH was approximately pH 4.6 and significantly less FAN was released at pH 4.0 or below and at pH 5.0 There is little information concerning the pH optima of the

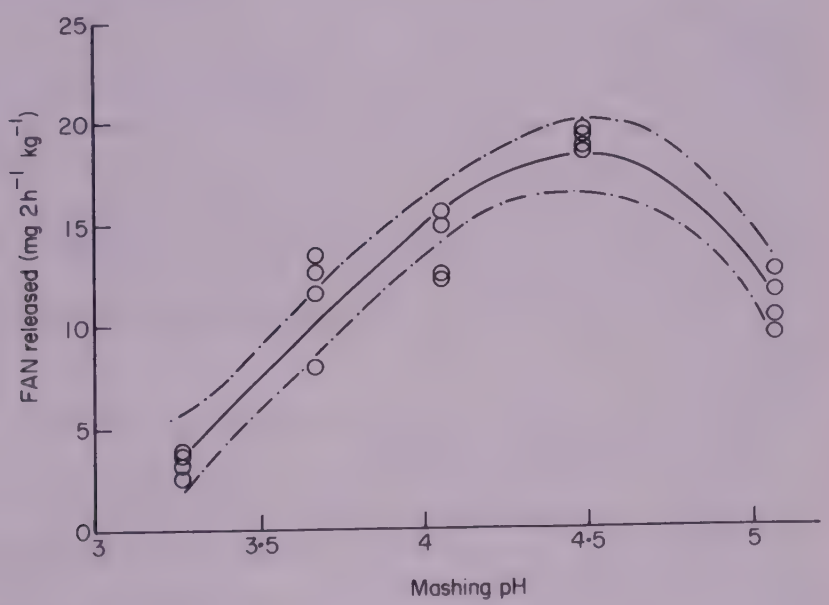

Figure 5. Effect of mashing pH on FAN production. Mashing carried out using an NK283 malt, FAN content 102 mg 100 g^{-1}: (—·—) 95% confidence limits.

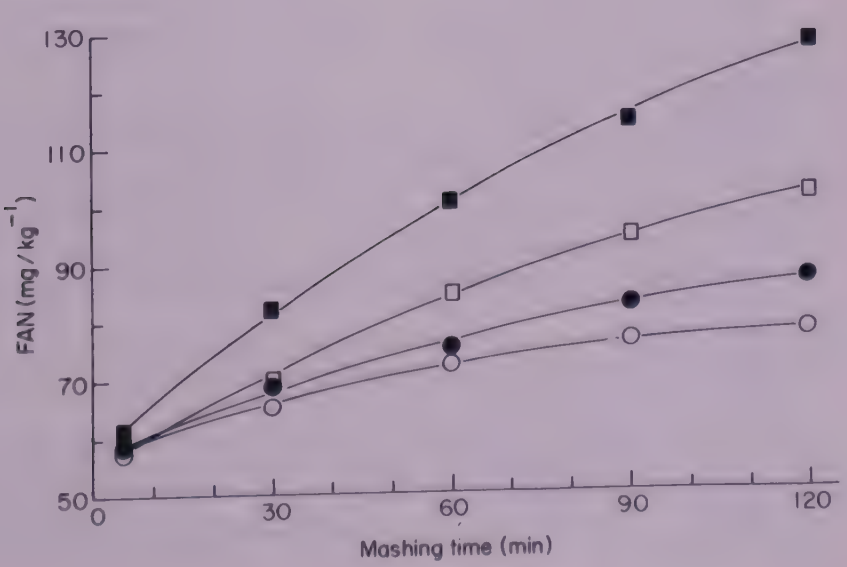

Figure 6. Effects of Milezyme AFP on FAN production during mashing. Mashing carried out using an NK283 malt, FAN content 102 mg 100 g^{-1}. Milezyme AFP concentration (percentage dry matter in mash): (○), control; (●), 0.01; (□), 0.03; (■), 0.10.

proteolytic enzymes of germinating sorghum. The purified enzyme discussed above exhibited maximum activity around pH 3.6 and showed negligible activity above pH 5.0.[15] Other studies have used an assay pH of 5.0.[16, 17] The proteolytic enzymes of germinating barley have been studied in much greater detail and both the proteinases[19] and carboxypeptidases[20] have acidic pH optima.

A further method of increasing FAN production during mashing is by the use of proteolytic enzymes of microbial origin.[18, 21] Figure 6 shows the effect of the addition to the mash of various concentrations of Milezyme AFP. This is a proteinase enzyme of fungal origin with an acidic pH optimum. It can be seen that FAN production was proportional to enzyme concentration. At the highest enzyme concentration, 0.1% of dry material in the mash, the FAN at the end of mashing was double that of the control. The actual release of FAN during mashing was 3.7 times greater than the control. However, before this enzyme can be used in brewing further research is required to determine whether the FAN produced supports normal yeast growth during fermentation.

4. Conclusions

This study has shown that under normal conditions of sorghum beer mashing 60°C, pH 4.0, approximately 30–35% of wort FAN is produced during mashing. The quality of the wort FAN is good compared to that of worts from barley malt. Wort FAN can be increased by a number of methods: increasing the ratio of malt to adjunct, reducing the mashing temperature to 51°C, increasing mash pH to 4.6, and by the use of microbial proteolytic enzymes. However, these methods all have disadvantages. They either involve additional expense in the case of using more malt or enzymes, or may adversely affect the quality of the beer by reducing sugar production or affecting viscosity when mashing conditions are altered. Probably the best method of ensuring that the wort contains sufficient FAN for good yeast growth is by mashing with malts containing good levels of FAN. The FAN content of the wort is directly proportional to that of the malt. Mashing with high FAN malts has the advantage that it involves no additional raw materials or changes in process. Concerning the attainment of high FAN sorghum malts, recent research has shown that malt FAN can readily be manipulated by modifying malting conditions.[22]

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Presence of Quercetin-3-*O*-Glucuronoside in Spanish Table Wines

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Quercetin-3-*O*-glucuronoside has been identified in several Spanish table wines by thin-layer (t.l.c.) and high-performance liquid (h.p.l.c.) chromatographic methods. This is thought to be the first report of its occurrence in wine.

Keywords: Flavonols; h.p.l.c.; t.l.c.; wines.

1. Introduction

Flavonols have been known to be in grapevine leaves, grapes and wines for a long time.[1] Several investigators have reported the presence of free flavonol aglycones in different wines,[2–9] but only a few of them have recognised the occurrence of flavonol glycosides.[6, 9–12]

Grape skins contain several 3-glycosides, and quercetin occurs in combination with glucuronic acid also, but free aglycones are absent.[7] On the other hand, wines appear to be relatively rich in free flavonol aglycones in relation to flavonol glycosides, probably as a consequence of their hydrolysis during fermentation.[1]

Although most flavonol glycosides reported in wines have also been found in grape skins, no data are available on the presence of quercetin-3-*O*-glucuronoside in wines. The aim of this work is to investigate the occurrence of this compound in some Spanish table wines from different regions.

2. Experimental

2.1. Wine samples

Three white and three red table wines from Valdepeñas (V-B and V-T), La Mancha (M-B and M-T) and Rioja (R-B and R-T), made in 1983, were used.

2.2. Chemicals

Chemicals used were as follows. Silica gel 60, microcrystalline cellulose (E. Merck), cellulose MN 300 G (Mächery Nagel), chromatographic paper (Whatman), diphenilboric acid 2-aminoethyl ester (Fluka), hydrochloric acid, methanol, chloroform, acetic acid, *n*-butanol and *t*-butanol (Panreac) of analytical grade were used for sample purification and column, paper and thin-layer chromatographic analyses. H.p.l.c.-grade solvents (Ferosa) were filtered through a 0.45 μm Millipore filter before h.p.l.c. Distilled, deionised water for h.p.l.c. was obtained from a Milli-Q water purification system (Millipore).

2.3. Isolation of quercetin-3-*O*-glucuronoside

A litre of wine was concentrated on a rotary evaporator at 35°C to about 50 ml. The concentrate was treated with 250 ml of 1% hydrochloric acid in methanol and filtered on a Büchner funnel, and the filtrate was concentrated on a rotary evaporator at 35°C to about 50 ml. This concentrate was percolated through a column packed with microcrystalline cellulose and deactivated silica gel,[13]

washing with *t*-butanol–acetic acid–water (3:1:1 by vol) and collecting the first 500 ml. The effluent was concentrated on a rotary evaporator at 35°C to about 10 ml, and then purified on thin layers of cellulose MN to 300 G (20×20 cm) with acetic acid–water (1:1 by vol) to separate flavonol aglycones (R_F=0.25) and flavonol glycosides (R_F=0.70). Flavonol glycosides were dissolved with methanol and chromatographed on Whatman 3MM paper, using distilled water as solvent, in order to isolate quercetin-3-*O*-glucuronoside (R_F=0.46) from other flavonol glycosides (R_F=0.12).[14] The purification of quercetin-3-*O*-glucuronoside was carried out in the last chromatographic system. Flavonol spots were monitored with ultraviolet (u.v.) light (360 nm).

2.4. Identification of quercetin-3-*O*-glucuronoside

Two-dimensional chromatography was carried out on thin layers (20×20 cm) of cellulose MN 300 G. Chromatograms were developed in three different solvent systems (Table 1). A one per cent diphenylboric acid 2-aminoethyl ester solution in methanol was used as spray reagent for detection of quercetin-3-*O*-glucuronoside. Spots were monitored at 360 nm u.v. light.

H.p.l.c. was performed on a Waters Associates liquid chromatograph, equipped with a Model 6000A solvent delivery system, a Model U6K injector, a Model RCM radial compression module, a Model 440 absorbance detector and a Model 730 data module. A Novapak C_{18} radial compression cartridge (Waters Associates), 10 cm×8 mm i.d., and a mobile phase of tetrahydrofuran–acetic acid–water (17.5:2.5:80), at a flow rate of 2.0 ml min^{-1}, were used throughout this study. Detection was carried out at 365 nm, with a detector sensitivity of 0.1 a.u.f.s. Samples (50 μl) were injected through a Microliter 805 syringe (Hamilton).

For t.l.c. and h.p.l.c. analyses, quercetin-3-*O*-glucuronoside isolated from *Vitis vinifera* leaves[15] was used as reference standard. The characterisation of this substance was carried out by chromatographic and spectrophotometric techniques, and also by acid hydrolysis.[14]

3. Results and discussion

A typical thin-layer chromatogram of quercetin-3-*O*-glucuronoside isolated from wine showed a sole dark purple spot in u.v. light, that changed to orange after spraying with 1% diphenylboric acid 2-aminoethyl ester solution in methanol. The t.l.c. constants of quercetin-3-*O*-glucuronoside isolated in the different wines analysed and those of the reference standard in the three chromatographic systems used are given in Table 1.

H.p.l.c. analyses of quercetin-3-*O*-glucuronoside isolated from the six wines showed a single peak, with retention times between 6.73 and 6.82 minutes. H.p.l.c. data of these samples and those of the reference standard are listed in Table 1.

T.l.c. and h.p.l.c. analyses showed the presence of quercetin-3-*O*-glucuronoside in the six wines analysed, although red wines appear to be richer than white wines, as could be expected, since

Table 1. Thin-layer chromatography constants (R_D×100) and high-performance liquid chromatography data of quercetin-3-*O*-glucuronoside

	Two-dimensional t.l.c.						H.p.l.c.	
	System 1		System 2		System 3			
Sample	BAW	AcH15	CAW	AcH15	TBA	AcH15	Retention time (min)	Capacity factor
Reference standard	56	53	4	52	54	53	6.75	2.81
White wine V-B	54	55	5	49	54	55	6.73	2.84
White wine M-B	57	54	5	49	56	54	6.78	2.81
White wine R-B	56	53	5	49	55	54	6.80	2.78
Red wine V-T	57	53	5	50	55	53	6.75	2.81
Red wine M-T	57	54	5	50	54	52	6.82	2.79
Red wine R-T	54	53	4	52	55	52	6.77	2.80

BAW: n-butanol–acetic acid–water (4:1:5, upper phase); CAW: chloroform–acetic acid–water (30:15:2, lower phase); TBA: *t*-butanol–acetic acid–water (3:1:1); AcH15: acetic acid–water (15:85).

flavonol glycosides are largely localised in the relatively solid part of the grape.[1] The amount of this compound is very small in wines, probably less than 1 mg litre^{-1}, and it has not been possible to carry out an adequate quantitative estimation in this laboratory.

4. Conclusions

The results prove the presence of quercetin-3-*O*-glucuronoside in several Spanish table wines, as could be expected, because this compound is a typical component of grape and its hydrolysis during fermentation appears to be more difficult than those of flavonol glycosides. The presence of this substance in the six wines analysed, which have been made with distinct technologies using different grape cultivars, suggests that this compound can be considered a typical component of wines.

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Saponin Content of Chickpea and Black Gram: Varietal Differences and Effects of Processing and Cooking Methods

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Differences were observed in saponin contents of eight varieties of chickpea (*Cicer arietinum*) and four of black gram (*Phaseolus mungo*). Common domestic processing and cooking treatments reduced the saponin level of the pulses significantly. Sprouting had the most pronounced effect followed by autoclaving, soaking and ordinary cooking. Cooking of soaked as well as unsoaked seeds had a similar diminishing effect.

Keywords: Saponin; chickpea; black gram; processing; cooking.

1. Introduction

Food legumes are known to contain substantial amount of saponins.[1,2] Saponins have been shown to possess a plasma cholesterol lowering effect in experimental animals.[3–6] If this effect also occurs in human beings saponin may have considerable physiological significance in reducing the risk of heart disease[6–8] in people having pulse in their diet.

Chickpea and black gram are two important pulses consumed by a large stratum of the Indian population. Legume grains are processed and consumed in a variety of forms. The most common methods of processing include soaking the seeds overnight, ordinary cooking or pressure cooking of soaked seeds, sprouting, cooking of sprouts and cooking of unsoaked seeds.

It is of interest to know the effect of these treatments on the saponin content of pulses. In the present study seeds of eight varieties of chickpea (*Cicer arietinum*) and four of black gram (*Phaseolus mungo*) were subjected to various domestic processing and cooking treatments and their saponin contents were determined.

2. Experimental

2.1. Materials

Seed samples of eight high yielding varieties of chickpea (C-235, H-208, H-78-65, H-77-66, H-76-67, H-75-35, H-81-73, H-82-2) and four of black gram (T-9, UH-80-7, Pant-U-30, Pant-U-19) were obtained from the Department of Plant Breeding, Haryana Agricultural University, Hisar (India). These varieties were grown under similar soil and agroclimatic conditions. Seeds were cleaned and freed from broken seeds, dust and other foreign material.

2.2. Processing and cooking methods

2.2.1. Soaking

Seeds were soaked in plain water for 12 h at 37°C. A seed to water ratio of 1:5 (wt:vol) was used. The unimbibed water was discarded. The soaked seeds were rinsed twice in distilled water and then dried in hot air oven maintained at 55°C (except those used for cooking and germination).

2.2.2. Cooking

The soaked seeds after rinsing in distilled water were put in round-mouthed tall beakers fitted with condensers connected to running water. After adding tap water (three times the weight of dry seeds),

the samples were cooked on a hot plate until they became soft as felt between fingers. Similarly, unsoaked seeds after keeping a seed to water ratio of 1:7 (wt:vol) were cooked in the same beakers till the seeds became soft. For pressure cooking, the soaked seeds were cooked at 1.05 kg cm^{-2} pressure for 15 min in an autoclave. For this, dry seeds to water ratio of 1:2 (wt:vol) was used. The cooked samples were mashed and then dried at 55°C.

2.2.3. Germination

The soaked seeds were germinated in sterile Petri dishes lined with wet filter paper in a BOD incubator kept at 25°C. To obtain a sprout measuring 1.5 to 2.5 cm, the usual size consumed, the seeds of chickpea and black gram varieties were germinated for 68 h and 48 h, respectively. The sprouts were rinsed in distilled water and dried at 55°C or the rinsed sprouts were cooked till soft, mashed and dried at 55°C.

The oven dried processed as well as unprocessed pulse samples were ground in a cyclone sample mill using 0.5 mm sieve and stored in plastic containers at room temperature until required for further analysis.

2.3. Saponin estimation

The method of Gestetner *et al.*[9] designed for determining soya bean saponin was followed. The ground pulse sample was refluxed with 0.5 M sulphuric acid in dioxane:water (1:3, by vol) to hydrolyse the saponins. The sapogenins so liberated were extracted with diethyl ether and separated on an alumina column. Sapogenin was determined colorimetrically using modified Liebermann–Burchard reagent (acetic acid: sulphuric acid; 3:2 by vol). The standard saponin was obtained from Sigma Chemical Co., Saint Louis, USA. The data were statistically analysed for analysis of variance.[10]

3. Results and discussion

3.1. Varietal differences

Saponin content of unprocessed seeds of the varieties of both the legumes is quite high and varied from 3952 to 4197 mg 100 g^{-1} in chickpea (Table 1) and 2700 to 2853 mg 100 g^{-1} in black gram

Table 1. Effect of domestic processing and cooking on saponin contents of chickpea[a]

Treatment	Variety							
	C-235	H-208	H-78-65	H-77-66	H-76-67	H-75-35	H-81-73	H-82-2
Soaking	3743±23 (10)	3713±23 (6)	3597±73 (10)	3565±43 (10)	3795±41 (5)	3780±43 (10)	3833±39 (6)	3720±36 (8)
Cooking of soaked seeds	3510±27 (15)	3347±96 (15)	3333±66 (16)	3303±82 (17)	3397±36 (16)	3605±45 (14)	3440±45 (15)	3407±35 (15)
Cooking of unsoaked seeds	3518±39 (15)	3458±65 (13)	3478±75 (13)	3460±65 (13)	3400±31 (15)	3637±41 (14)	3587±41 (12)	3602±18 (11)
Autoclaving of soaked seeds	2693±97 (35)	2413±74 (39)	2572±68 (36)	2447±62 (38)	2652±53 (34)	2628±16 (38)	2558±39 (37)	2718±47 (33)
Sprouting of soaked seeds	2308±83 (44)	2028±41 (49)	2093±82 (47)	2153±118 (46)	2197±79 (49)	2292±59 (46)	2073±72 (49)	1988±62 (51)
Cooking of sprouted seeds	1857±48 (55)	1497±47 (62)	16832±85 (58)	1563±113 (60)	1253±20 (69)	1442±39 (66)	1792±55 (56)	1373±41 (66)
Control (unprocessed seeds)	4138±36	3952±128	3980±15	3967±27	3992±46	4197±58	4057±55	4025±41

[a] Values, means±s.d. of six independent determinations, are expressed on dry matter basis as mg 100 g^{-1}. Figures in parentheses represent loss in saponin content expressed as percentage of control values. c.d. ($P<0.05$)=160

Table 2. Effect of domestic processing and cooking on saponin contents of black gram[a]

Treatment	Variety			
	T-9	UH-80-7	Pant-U-30	Pant-U-19
Soaking	2572±17 (10)	2538±48 (6)	2563±50 (10)	2678±7 (6)
Cooking of soaked seeds	2385±44 (16)	2275±36 (16)	2467±34 (13)	2575±37 (10)
Cooking of unsoaked seeds	2462±11 (14)	2318±67 (14)	2523±55 (11)	2648±37 (7)
Autoclaving of soaked seeds	2242±47 (22)	2155±45 (20)	2077±83 (27)	2127±54 (25)
Sprouting of soaked seeds	1803±34 (37)	1637±56 (39)	1698±34 (40)	1753±23 (38)
Cooking of sprouted seeds	1472±60 (49)	1357±48 (50)	1422±26 (50)	1480±73 (48)
Control (unprocessed seeds)	2853±28	2700±45	2845±35	2842±30

[a]Values, means±s.d. of six independent determinations, are expressed on dry matter basis as mg 100 g^{-1}.

Figures in parentheses represent loss in saponin content expressed as percentage of control values.

c.d. ($P<0.05$)=90.

(Table 2). Similar differences in the saponin content of different cultivars have been observed in navy beans[2] and moth beans.[11] The results uphold the earlier report that food legumes are one of the most significant sources of saponins in man's diet.

3.2. Effect of processing and cooking methods

Soaking in plain water for 12 h reduced the saponin contents to a similar extent in all the varieties of both the pulses (Tables 1 and 2). Ordinary cooking of soaked and unsoaked seeds of both kinds reduced the saponin still further. Autoclaving of soaked seeds caused a greater reduction in saponin levels ranging from 33–39% in chickpea and 20–27% in black gram. Of all the treatments sprouting appeared to have the most severe effect on saponins. Losses of 44–51% and 37–40% were observed in chickpea and black gram, respectively. Cooking further decreased the saponin level of sprouted seeds. The sprouted seeds appeared to show the greater loss after cooking but this was cumulative effect accruing from soaking, germination and cooking. The major loss was caused by germination but cooking increased the total saponin lowering effect when it followed sprouting.

The loss of saponins from the seeds during soaking may be due to leaching while loss during cooking and autoclaving may indicate the thermolabile nature of saponins. Whether heat processing of seeds results in the formation of poorly extractable complex between saponin and sugars or amino acids is not known. Enzymic degradation is a possible explanation of the saponin loss during germination, but this has yet to be established. In a similar study, Khokhar and Chauhan[11] reported loss of saponin from moth bean seeds upon soaking in plain water or mixed mineral salt solution, ordinary or pressure cooking of soaked seeds and sprouting and cooking of sprouted seeds. The findings of this study, however, contradict the earlier report[2] that saponins survive the rigors of cooking and food processing.

The study concludes that chickpea and black gram are rich sources of saponins and can contribute substantially to saponin content of human diet. Common methods of domestic processing and cooking, to which these pulses are subjected before they are consumed, decrease the saponin level of the seeds to a varying extent. Germination has the most pronounced saponin lowering effect followed by pressure cooking, soaking and ordinary cooking.

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Effects of Wheat Proteins on the Viscoelastic Properties of Starch Gels

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The interactions between starch and wheat proteins were studied in oscillatory rheological measurements. The starch varieties examined were maize, barley, wheat, triticale, rye and potato. The storage modulus, G', and the phase angle, δ, were found to be most sensitive to the composition of the starch gel. When starch was gelatinised in presence of gluten it was found that G' increased for wheat and rye starches, whereas the presence of gluten proteins caused the opposite effect to maize starch. The viscoelastic behaviour of the other starches was not affected by the presence of gluten. A water soluble wheat protein fraction had the same effect on the viscoelastic behaviour of wheat starch as gluten, whereas ligolin behaved like a polar lipid.

Keywords: Starch gels; cereals; protein-starch interaction; gluten; lipid; rheology; viscoelasticity.

1. Introduction

Starch is the major component in cereal based foods like, for example, bread. Although starch is the major component, the baking quality of a wheat flour is attributed to proteins and lipids, and not to the starch.[1–3] However, baking experiments have shown that only wheat, rye and barley starches perform well in baking, whereas all other starches, for example potato, rice, oat and maize, give very poor results.[4] There seems to be no simple relation between the baking results and gelatinisation temperature or starch granule size. It could thus be speculated that there is a specific interaction between the starch granules from wheat, barley and rye, respectively, and the gluten matrix.[5] Starch–protein interactions involving the water-soluble proteins have also been suggested as an explanation for the differences between hard and soft wheat endosperms.[6] Further, both starch and gluten are frequently used as additives in the food industry, and thus interactions between wheat proteins and starch might be of importance for the quality of food products. The aim of the present investigation was to study this type of interaction.

To study interactions between starch and proteins, for example interactions between potato starch and fish proteins,[7] rheological methods and differential scanning calorimetry (d.s.c.) have been used. Differential scanning calorimetry has also been used to study the effect of gluten on the gelatinisation of wheat starch.[8] The interaction between gluten and wheat starch has also been examined by measuring iodine binding capacity of amylose and amylopectin in the presence of gluten.[9] In the present investigation the oscillatory rheological method was used to study starch–protein interactions. The behaviour of starch during gelatinisation and of gelatinised starches has been thoroughly investigated by rheological methods.[10] These include empirical as well as fundamental methods. Usually the starch gels have been studied under great deformation, which causes a lot of damage to the gel structure. When smaller amplitudes are used, for example in an oscillation test,[11, 12] a better opportunity to study interactions between starch and other components is obtained. It has also been shown that, from a rheological point of view, gluten (and modified gluten) might be regarded as a single component[13, 14] which simplifies the interpretations of results obtained when gluten is added to starch. In order to elucidate whether there is a specific wheat starch–wheat protein

interaction or if there is a general starch–gluten interaction several starches were included in the present investigation. The wheat proteins studied were both storage proteins (gluten) and water soluble proteins.

2. Experimental

2.1. Materials

Maize (CPC 03403), potato (Furina Potato Starch Extunnel AVEBE) and wheat (Tenstar Aquitaine A-starch) starches used were of commercial quality. Samples of wheat (Amy), rye (Petkus II), barley (Jenny) and triticale (6015) were supplied by Svalöf AB, Svalöv, Sweden, and the starch was extracted from the grains as described below. The monoglycerides used were saturated monoglycerides (Dimodan PVP, Grindsted Products A/S, Brabrand, Denmark). The soya bean oil was received from AB Karlshamns Oljefabriker, Karlshamn, Sweden. The gluten used was of commercial quality (Raisio, Finland) except in one experiment when gluten prepared from wheat flour of the variety Amy was used.[8] The wheat protein fraction ligolin was prepared from flour of standard baking quality exactly as described by Frazier *et al.*[15] but without any radioactive label. The final preparation result was verified with gel chromatography according to Frazier *et al.*[15] The other wheat protein fraction, the water soluble fraction, WSF (extracted with water from the wheat variety Monopol), has been described elsewhere (L. Lindahl, to be published).

2.2. Preparation of starch gels

Starch was extracted from grains as described by Meredith *et al.*[16] From this preparation the A-fraction was used. All the starch gels were made in a concentration of 6.5–6.6% (by wt) on dry weight basis. Proteins or lipids were added to give a concentration of 1% (by wt) calculated on the dry starch content. In the case of wheat starch, gluten was also added at 2 and 4% (by wt) levels. Starch and proteins or oil were mixed dry in a test tube before the water was added. The monoglyceride was preheated to 70°C at a monoglyceride:water ratio of 1:12 and then mixed with the starch/water suspension. The heating of the starch suspensions was performed according to Wong and Lelievre.[12] The capsulated test tubes were carefully shaken and immediately placed in a 95°C waterbath for 30 s. The shaking procedure was then repeated and the test tube was replaced in the waterbath. After 1 h at 95°C, the test tube was transferred to a bath at 30°C and equilibrated at this temperature for 30 min. The rheological measurements were performed during the next 30 min.

2.3. Measurement of rheological properties

The dynamic viscoelastic behaviour of the starch gels was measured in the Bohlin Rheometer System (Bohlin Reologi, Science Park Ideon, Lund, Sweden). A cone-plate system, with the radius of 15 mm and an angle of 5.4° between cone and plate, was used. The oscillation was performed between 0.02–1.0 Hz with a rotating angle of 0.413°. All experiments were made at a temperature of 25±0.5°C. Every single measurement was completed within 5 min, and was repeated at least three times with a deviation of 6% or less. The mean value from these tests is shown in the figures. The time from the start of the heating of the starch suspension until the measurements were concluded never exceeded 2 h.

3. Results

3.1. Starch gels

When rheological measurements are carried out in oscillation mode, it is possible to receive values of the storage modulus, G', the loss modulus, G'', the dynamic viscosity, $\eta = G''/\omega$, and the phase angle, $\tan \delta = G''/G'$. The frequency-dependence of these four parameters is shown in Figure 1 for a wheat starch gel, and in Table 1 these parameters are given at 0.1 Hz for all the starch gels investigated. It is evident from Table 1 that G' was most sensitive to the composition of the starch gel, and thus only the G'-values together with the phase angle are presented in the following discussion. The frequency-

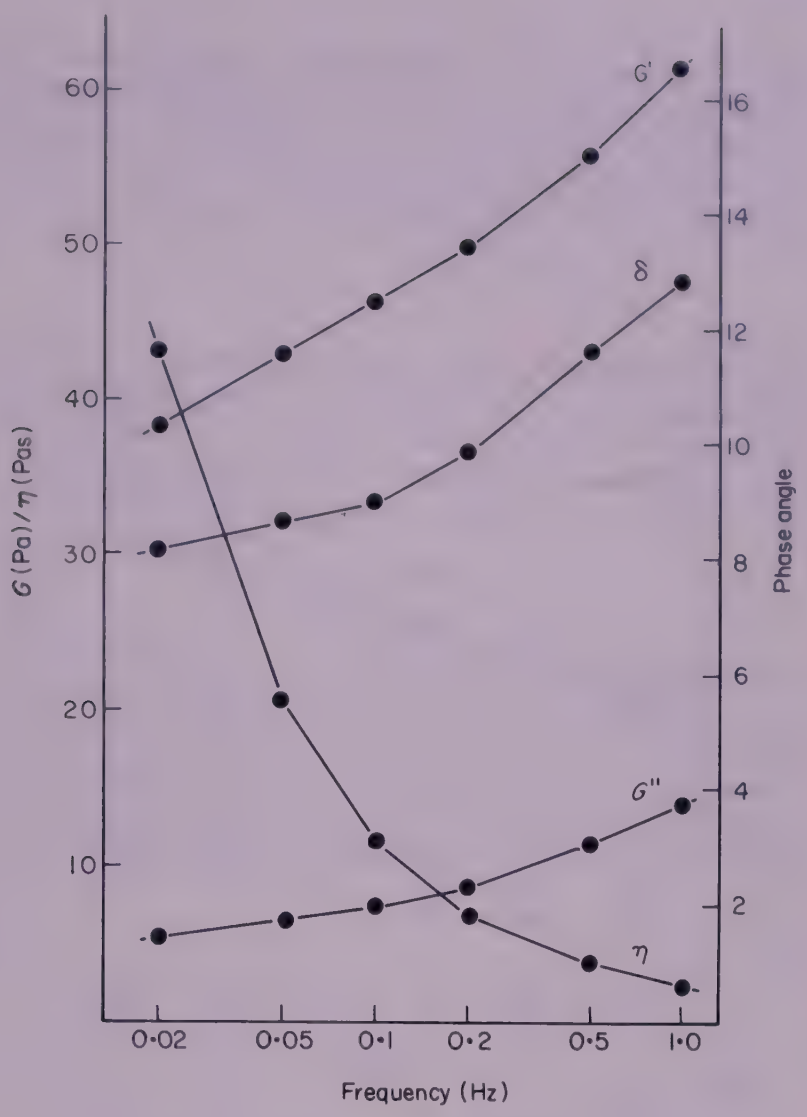

Figure 1. The frequency-dependence of the storage modulus, G', the loss modulus, G'', the dynamic viscosity, η, and the phase angle, δ, for a 6.6% (by wt) wheat starch gel (Amy).

dependence of G' and η observed for wheat starch (Figure 1) was very similar to the results obtained for wheat starch by Wong and Lelievre.[12] The frequency-dependence of G' for all the starches investigated is given in Figure 2 and these results show that great differences in G' existed between the starches studied. In maize starch the G'-value was about twice the value for the other cereals, or even more compared to rye. The similarity between starch from triticale and wheat starch indicated that in this triticale sample the wheat properties dominated. It is also notable that rye starch gave such a different rheological response compared to wheat, triticale and barley starches.

3.2. Starch gels with polar lipids

The interaction between polar lipids and starch is well-known and the effects of polar lipids on the rheological properties of starch have been demonstrated in such instruments as the Brabender

Table 1. The storage modulus, G', the loss modulus, G'', the phase angle, δ, and the dynamic viscosity, η, at the frequency 0.1 Hz for different starch gels

Starch	G' (Pa)	G'' (Pa)	δ	η (Pa)
Maize	141	7.9	3.2	12.6
Barley	53.9	10.4	10.9	16.5
Wheat (Amy)	46.5	7.3	8.9	11.6
Wheat (commercial)	39.8	7.9	11.3	12.6
Triticale	45.7	5.2	6.5	8.3
Potato	16.7	5.5	18.3	8.7
Rye	11.3	4.5	22.2	8.2

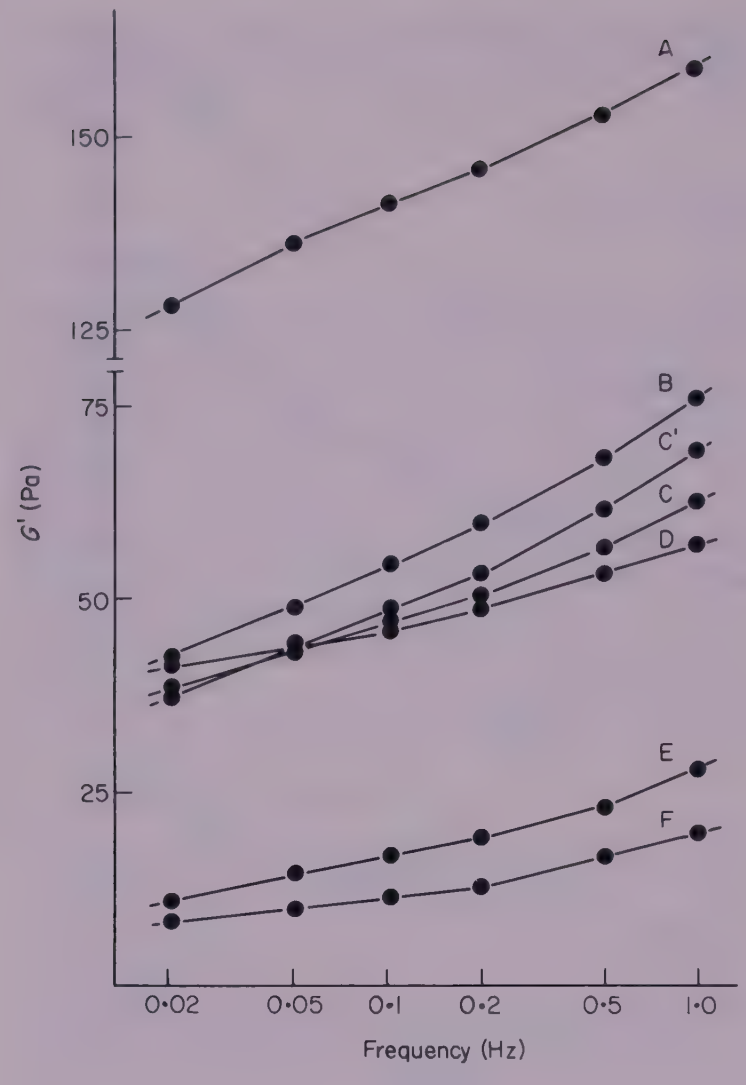

Figure 2. The storage modulus, G', as a function of the frequency for starch gels. A=maize, B=barley, C=wheat (Amy), C′=wheat (commercial quality), D=triticale, E=potato, F=rye.

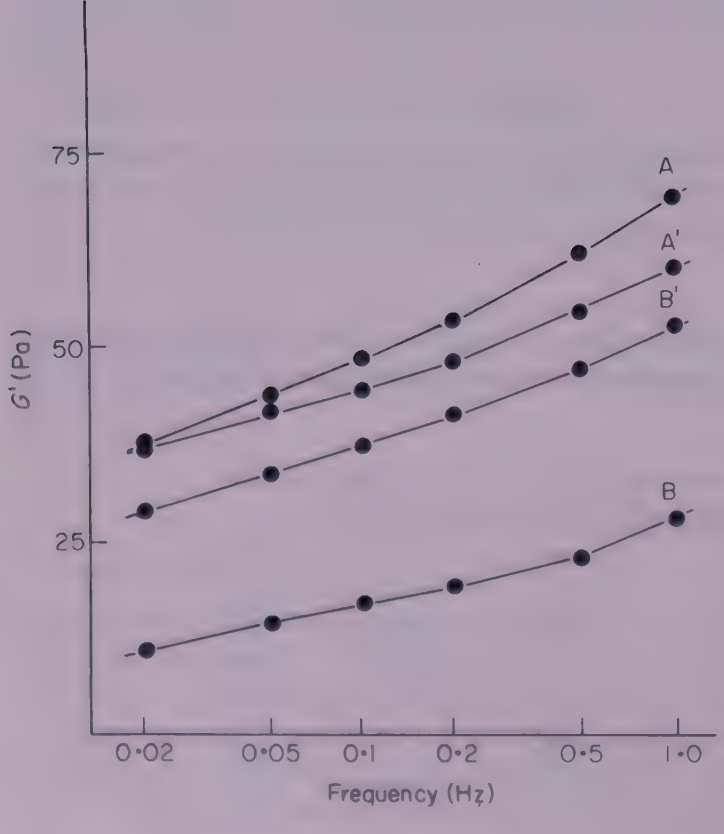

Figure 3. The effect of saturated monoglycerides (SMG) on the storage modulus, G', for starch gels. A=wheat starch (commercial quality), A′=wheat starch+1% SMG, B=potato starch, B′=potato starch+1% SMG.

Visco/Amylograph.[17] Before starch–protein interactions were studied it was established that the starch–lipid interaction could be observed in the present method. Saturated monoglycerides (SMG) were added to wheat and potato starches and the effects on the G' values are shown in Figure 3. The addition of SMG resulted in great changes in G' for both starches, although the changes were quite opposite to each other. G' increased to the double value in the case of potato starch, whereas it decreased in the case of wheat starch.

3.3. Starch gels with gluten

The effects of gluten on the storage modulus of starch are presented in Figure 4. Gluten gave in these experiments a lowering of G' for maize starch, and an increase in G' for wheat and rye starches. The greatest increase was obtained for rye starch, where G' was almost doubled. The effect of gluten on barley, triticale and potato starches was negligible. This behaviour is in the case of triticale somewhat difficult to understand as triticale is a genetic mixture of wheat and rye, and both these starches were clearly affected by the presence of gluten.

3.4. Wheat starch gels with wheat proteins

Studies with different amounts of gluten added were performed for wheat starch, and it was found that G' increased with increasing levels of gluten (Table 2). At the highest level (4%), however, it was difficult to obtain a homogeneous mixture of starch and gluten. At this level δ increased compared to the control, whereas at all other levels δ decreased. When gluten from the same variety as the starch (i.e. Amy gluten added to Amy starch) was tested as an additive, this addition resulted in higher elastic values. To obtain the same G' value 2% of the commercial gluten had to be added

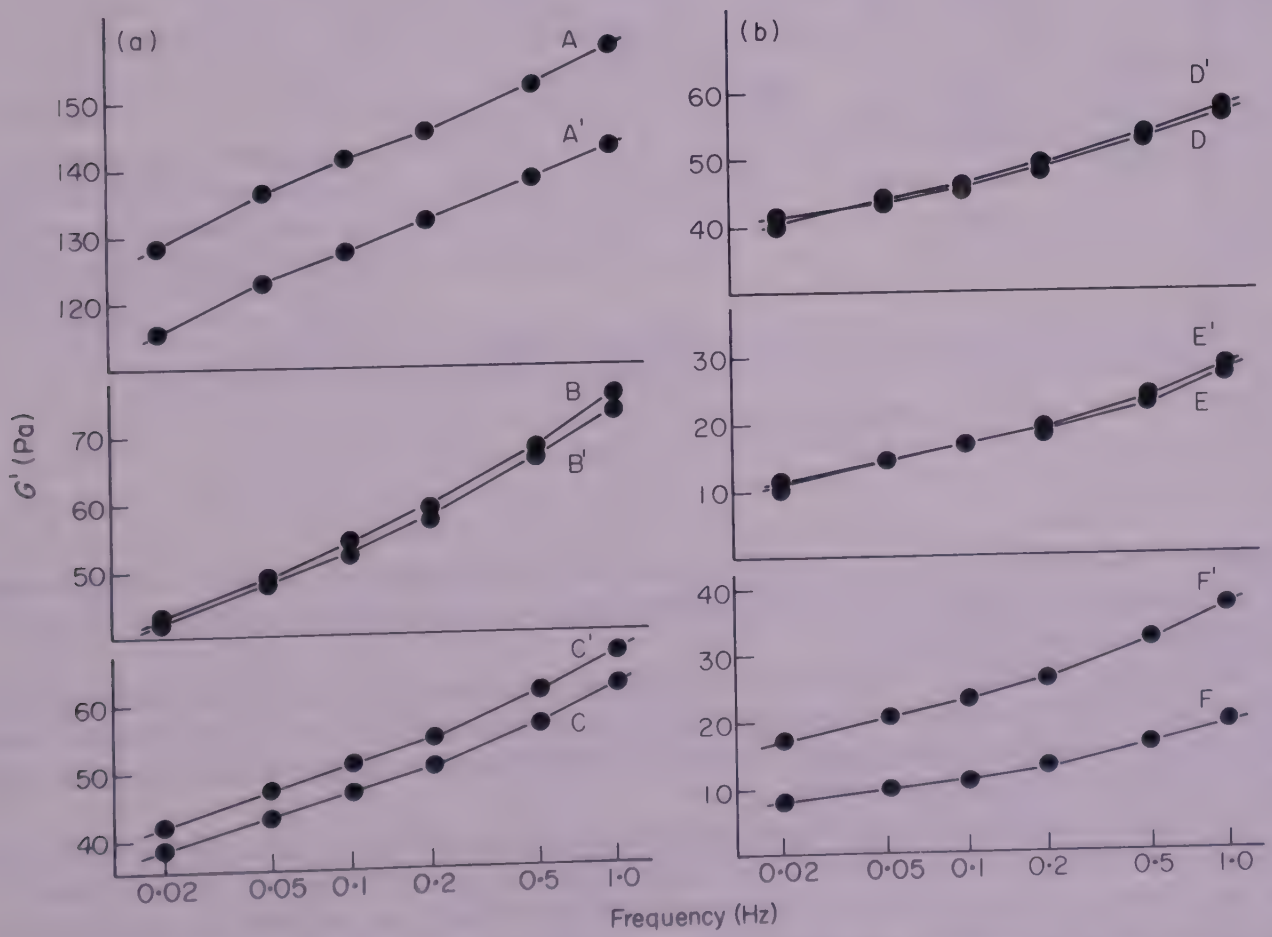

Figure 4. The effect of gluten on the storage modulus, G', for different starch gels. (a) A=maize starch, A'=maize starch+1% gluten, B=barley starch, B'=barley starch+1% gluten, C=wheat starch (Amy), C'=wheat starch+1% gluten. (b) D=triticale starch, D'=triticale starch+1% gluten, E=potato starch, E'=potato starch+1% gluten, F=rye starch, F'=rye starch+1% gluten.

Table 2. The storage modulus, G', and the phase angle, δ, at the frequency 0.1 Hz for wheat starch gels (6.6% Amy starch) with different amounts of gluten

Gluten (weight-% of dry starch)	G' (Pa)	δ
0	46.5	8.9
1	50.8	7.9
1[a]	53.7	8.1
2	53.9	8.2
4	57.3	9.3

[a] Amy-gluten.

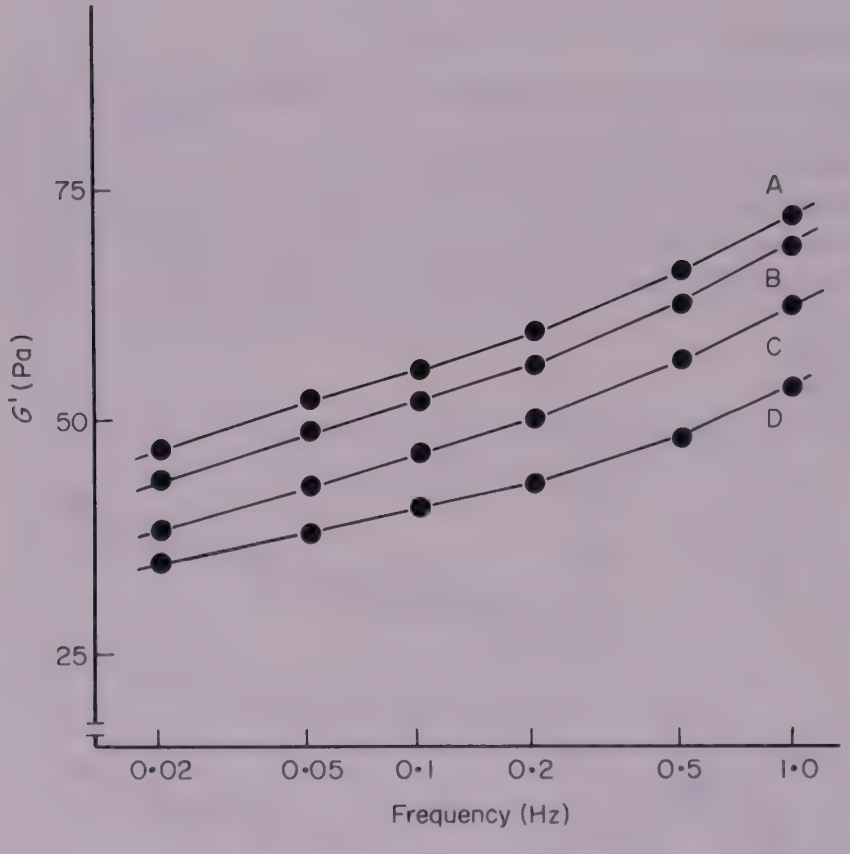

Figure 5. The effect of certain additives on the storage modulus, G', for the wheat starch Amy. A=wheat starch+1% soya bean oil, B=wheat starch+1% WSF, C=wheat starch without additives, D=wheat starch +1% ligolin.

(Table 2). Thus, not only the starch variety but also the protein variety seemed to be of importance for the interaction. However, in case of a commercial gluten the preparation procedure might also affect its interaction possibilities.

A water soluble fraction, WSF, from wheat flour was also used to examine the interaction with wheat starch. WSF consisted of rather small proteins, 65 000 and less in molecular weight, and of carbohydrates, mainly starch fragments. Addition of this mixture to wheat starch had an identical effect on the G' values as gluten (Figure 5).

As the wheat proteins and the monoglycerides affected the wheat starch gel in different ways it was thought to be of interest to study the wheat flour component ligolin. The ligolin fraction consists of one mole of triglyceride per mole of protein according to Frazier *et al.*[15] The material used was prepared as described in the literature[15] and gel filtration on Sephadex G-50 Fine (Pharmacia) showed a protein peak in the area of 10–12 000 in molecular weight. The effect of ligolin on the G' values of wheat starch was similar to the results obtained by adding SMG, i.e. G' decreased (Figure 5). The next experiment was done to investigate whether the lowered G' value depended on the

triglyceride or if it could be related to the protein present. Wheat starch was therefore mixed with corresponding amounts of soya bean oil. As can be seen in Figure 5 addition of triglycerides had the opposite effect to the monoglyceride addition. A mixture of gluten and soya bean oil was also added to wheat starch (not shown in the figures), and the gel behaved similar to the gel with only one of the components added.

4. Discussion

The starch gel might be regarded as a composite material composed of more or less swollen starch granules dispersed in a continuous polysaccharide matrix.[18] The rheological properties of such a material depend on properties of the dispersed starch granules, on properties of the continuous matrix and on interactions between the dispersed phase and the continuous phase. The differences observed between starch species, and the effects of additives like polar lipids or proteins, will be discussed in relation to this model.

During the gelatinisation the starch granules absorb water and swell, and polysaccharide material, preferentially amylose, leaches out from the granules. With a starch concentration between 6.5–6.6% (by wt) there is always a great excess of water present, which guarantees a complete gelatinisation of the starch.[19] The results in Figure 2 show that great differences in starch gel behaviour, depending on the nature of the native starch, exist. The large value of G' observed in pure maize starch (Figure 2) might be related to the starch granules being more rigid in this starch, whereas the low G' values observed for potato and rye starches consequently could be due to a lower rigidity of these granules. Microscopic investigations have shown that the swelling behaviour is quite different for these starches.[20] Further, the values of the phase angle (Table 1) show that a more elastic gel is formed by maize starch and by triticale starch, whereas potato and rye gave more viscous gels. The low δ values of maize and triticale starch gels might be due to a better granule–granule contact in these gels, which might result in the formation of a transient network in the gel.[21]

The effect of polar lipids on the rheological properties of starch gels is usually attributed to the formation of an amylose–lipid complex. The presence of such a complex might affect the starch gel in at least two ways: the dispersed particles might be more rigid due to a lesser degree of swelling in presence of for example SMG, and the interaction between the dispersed phase and the continuous phase might decrease when amylose molecules are involved in the complex formation. In potato starch evidently the first effect predominates (Figure 3), whereas in wheat starch the second effect predominates.

The interaction between proteins and starch could not be expected to occur in a way similar to the complex formation between amylose and polar lipids. Instead a kind of surface interaction between the granule and the gluten molecules can be responsible for the effects observed. Small amounts of proteins present on the surface of starch granules from certain species might be of importance for this interaction. An increasing effect of gluten on G' was clearly demonstrated for wheat and rye starch (Figure 4), and together with the decreasing phase angle observed for the wheat at low levels of gluten addition (Table 2) and also for rye starch gels (results not shown), this might indicate that gluten facilitates the granule–granule contact and thus enhances the transient network discussed above. As this effect was not observed in all starch gels, a necessary condition for the interaction might be that the surface of the starch granule has certain properties, for example, contains specific groups of proteins. Wheat starch granules are known to contain adhering material,[5, 22] and this might be the case also with the other cereal starches. Especially rye starch could thus be expected to contain the necessary groups in great amount as the same amount of gluten gave such a dramatic enhancement in G' value, almost 100%, in rye starch compared to about 10% in wheat starch. Barley and triticale starches, on the other hand, seemed to be unaffected by the presence of gluten, and it could be asked why triticale starch did not interact with gluten in a way similar to wheat and rye starches. One explanation could be that triticale (and barley) starch lacks the necessary groups on the granule surface, or that there are some specific groups on the surface that inhibit a probable 'binding' to gluten proteins. In the case of maize starch the addition of gluten gave a decrease in G' (Figure 4). The presence of gluten thus seemed to destroy the elastic properties of the maize starch gel. Potato

starch is known to be very pure,[23] and the starch granule surface could be expected to contain only phosphate groups and perhaps some lipids.[24] This might explain why no interaction seemed to occur between potato starch and gluten (Figure 4b). These components seemed to be incompatible as a separate phase of gluten was observed at the bottom of the test tube when potato starch and gluten were heated together. No such sedimentation could be observed for any of the other starches heated with gluten. However, these effects need to be further investigated in order to correlate the results with the presence of specific groups on the surface of starch granules from different species.

In view of the results obtained from wheat starch and gluten proteins, the similar behaviour obtained in the case of the water soluble fraction, WSF, could be expected. Especially since the WSF has a 'gluten-pattern' in its amino acid composition (L. Lindahl, to be published). The addition of ligolin was of special interest due to its composition of a triglyceride and a protein. As the result indicated a behaviour similar to a monoglyceride and not to an oil, it might be questioned whether there really is a triglyceride involved in the ligolin fraction when prepared directly from flour.

Acknowledgements

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Selenium Content of Wheat Flour Used in the UK

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The selenium content of a range of wheat flours used primarily for bread was determined. This was found to be significantly correlated ($P<0.001$) with the protein content of these flours. The selenium content of flour and bread in the UK was compared with previously determined values for the UK and with values quoted for North American flours and breads showing that the effect of the recent inclusion of a higher proportion of home-grown wheats was to lower human dietary selenium intakes markedly. These intakes are now calculated to be around 43 μg day^{-1} and consequently substantially below recommended daily intakes.

Keywords: Selenium; wheat flour; bread; protein; human RDA.

1. Introduction

The effects of the trace element selenium in farm animals are documented both in deficiency (white muscle disease in lambs and calves, exudative diathesis in poultry and hepatosis dietetica in pigs) and in excess (blind staggers and alkali disease).[1] The exact effects in man are less well established. Awasthi *et al.*[2] have shown that selenium is essential as a constituent of erythrocyte glutathione peroxidase (EC 1.11.1.9). Epidemiological evidence has been cited that links certain types of cancer with low selenium status.[3] Aetiological comparisons have also suggested a link between low selenium status and increased incidence of cardiovascular disease,[4] while severe selenium deficiency seems to be identified with endemic chronic cardiomyopathy—Keshans Disease—in China.[5]

The average intake of selenium in Britain is calculated as 60 μg day^{-1} by Thorn *et al.*[6] who pointed out that this is lower than quoted for Canada, 110–220 μg day^{-1},[7] and America, 100 μg day^{-1},[8] Cresta *et al.*.[9] quote 100 μg day^{-1} for the Netherlands, 166 μg day^{-1} for France and 141 μg day^{-1} for Italy with Japan estimated at 208 μg day^{-1}.[10] However, Belgium intake[11] is measured as 40–60 μg day^{-1} while New Zealand is variously quoted as 25 μg day^{-1},[12] 30 μg day^{-1} [13] and 56 μg day^{-1}.[14] Finnish dietary intake is among the lowest (30–35 μg day^{-1})[15] with areas of China where Keshans Disease is endemic being below 16 μg day^{-1}.[16]

Few recommended levels of selenium intake exist but the Food and Nutrition Board[17] of the National Academy of Science has recommended a daily intake of 50–200 μg for adults.

In light of the higher proportion of home-grown flour now being used in the UK in bread manufacture it seemed appropriate to measure the selenium content, compare the levels obtained with the protein content and consider the possible effect on regional selenium intake. The results obtained are presented and discussed in this paper.

2. Materials and methods

2.1. Source of flour samples

These were supplied by: Allied Mills Ltd, Glasgow; Bermaline Ltd, East Lothian; Campbell William & Co, Glasgow; Carr's Flour Mills Ltd, Carlisle; Gideon Walker Ltd, Edinburgh; Rank Hovis McDougall plc, Solent Mills, Southampton.

The samples represented flour in routine production and used in the baking of various types of bread from wholewheat to sliced pan. The bread samples were of leading brands, purchased locally.

2.2. Analytical

Selenium was determined by a modification of the fluorimetric procedure of Olson.[18] The sample was digested in a nitric/perchloric acid mixture[a] and the fluorescence measured on the piazselenol complex formed between the selenium and 2,3-diaminonaphthalene. The modification involved the use of 2,3-diaminonaphthalene hydrochloride which has the effect of lowering the blank values and hence of increasing the sensitivity of the method. Protein was determined by modified Kjeldahl.

2.3. Diet survey

The weights of different foods consumed on average in different regions of the UK were abstracted from the Ministry of Agriculture, Fisheries and Food (MAFF), National Food Survey (1982).[19] The region designated 'Scotland' in this survey was composed of data gathered in the four political constituencies: East Kilbride, Glasgow Garscadden, Central Dumbartonshire and East Aberdeenshire. Permission was granted by MAFF to consult the unpublished data from 'East Kilbride' and 'Glasgow Garscadden'.

3. Results

Table 1 shows the range of selenium content in flour used in bakeries in the UK and Figure 1 shows the correlation of selenium content with protein. The selenium concentrations ranged from very low values of 0.023 μg g^{-1} for low protein flour to high values (>0.5 μg g^{-1}) for high protein flour. Subdivision of the high protein flours revealed that brown and wholewheat flours never fell below 0.267 μg Se g^{-1} whereas white flour of similar protein content fell to 0.126 μg Se g^{-1}.

Table 1. Selenium content of flours (μg g^{-1}) used in UK bakeries

Flour	Number of samples	Mean Se concentration	s.e.	Range
a. Protein content <12%	8	0.048	0.009	0.023–0.098
b. Protein content 12–14%	8	0.163	0.029	0.039–0.224
c. Protein content >14%	28	0.338	0.026	0.126–0.576
White flours	17	0.327	0.037	0.126·0.576
Brown and wholewheat flours	11	0.356	0.033	0.267–0.566

All figures are on a dry weight basis.

Table 2 shows that the breads produced from low protein, medium protein and wholewheat flours on a fresh weight basis were lower in selenium content than the values found for the flours from which they had been baked but this is largely due to the difference in moisture content between bread and flour (38% and 10%).

In Figure 2 the weights of foods consumed according to the National Food Survey[19] are compared with the selenium content. The selenium content of foods other than those based on wheat flour are taken from the data of Thorn *et al.*[6] The wheat based foods were calculated using the data presented here. This would decrease Thorn's estimate of total selenium intake of 60 μg day^{-1} to around 43 μg day^{-1} and the proportion of selenium supplied by cereals from 50% to 42%.

4. Discussion and conclusions

In this country bread was formerly made from high protein (>14% CP in DM) imported Canadian flour. Recent information from the Home-grown Cereals Authority, however, indicated that the percentage of home-grown wheat used by flour millers in the UK had risen from 54% in 1979–1980 to

[a] The cited procedure should be followed carefully and with caution.

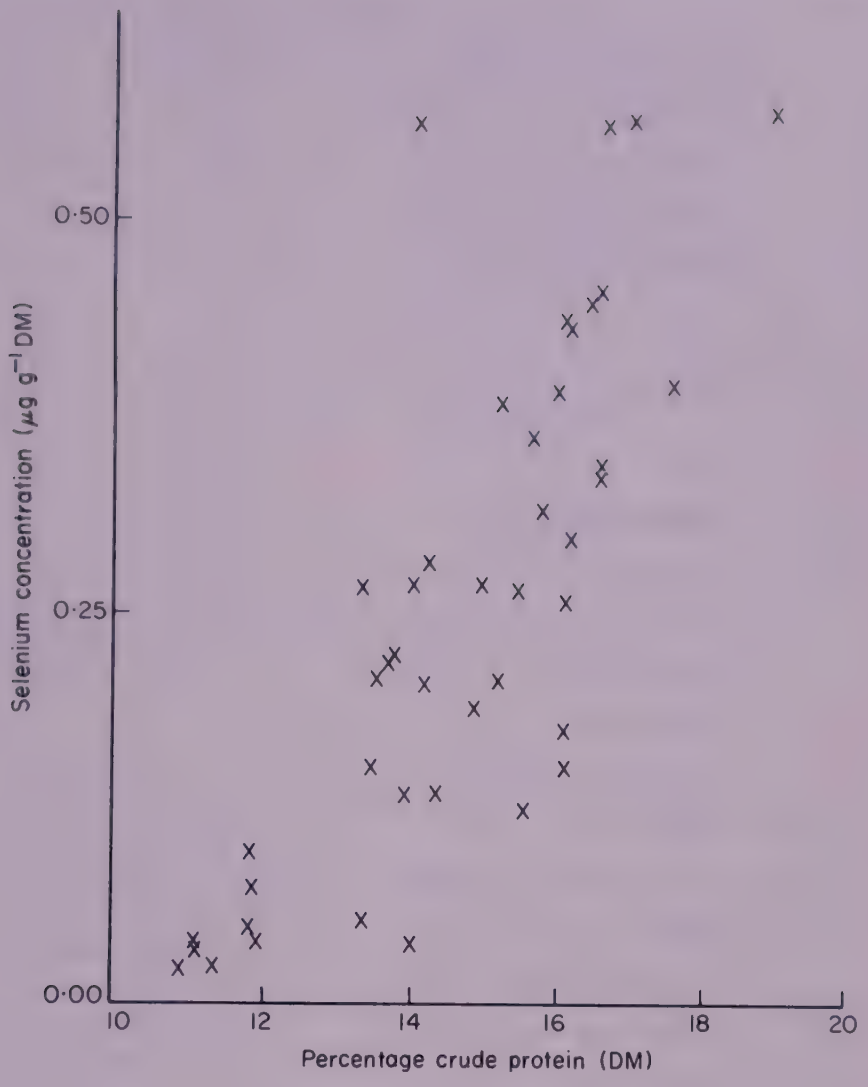

Figure 1. Relationship between crude protein (percentage DM) and selenium (μg g^{-1} DM) in flour: r=0.79

Table 2. Selenium content of bread (μg g^{-1})

	Number of samples	Selenium content		s.e.	
		Fresh wt	Dry wt	Fresh wt	Dry wt
Pan (from low protein flour)	3	0.036	0.057	0.0003	0.0005
Square white (from medium protein flour)	3	0.112	0.180	0.0006	0.0010
Wholewheat	3	0.188	0.314	0.0006	0.0010

82% in 1984–1985. The selenium content of wheat, like all plants, varies with its source of origin and reflects the selenium status of the soil on which it was grown.[20] Canadian wheat is known to be high in selenium.[21] This can be seen by reference to Table 3 which details the flour and bread selenium contents recorded by previous workers in Canada, the USA and the UK. Note, all data shown here are on a fresh weight basis. The reduction in the selenium contents of 'bread-making' and wholewheat flours found in the present study compared to those of Thorn *et al.*[6] will reflect the lower proportion of imported flour currently used in the higher protein grades. There has been no similar change in the selenium content of the softer white flour between the two studies which probably indicates that this has remained of predominantly UK origin.

As indicated in Table 3, on a fresh weight basis, bread selenium content was found to be lower than the flour from which it was produced. This is largely due to the difference in moisture contents of bread and flour as well as possibly to the inclusion of the other ingredients, although Higgs *et al.*[22] have reported that baking removed from 7–23% of the initial selenium content. This evidence is

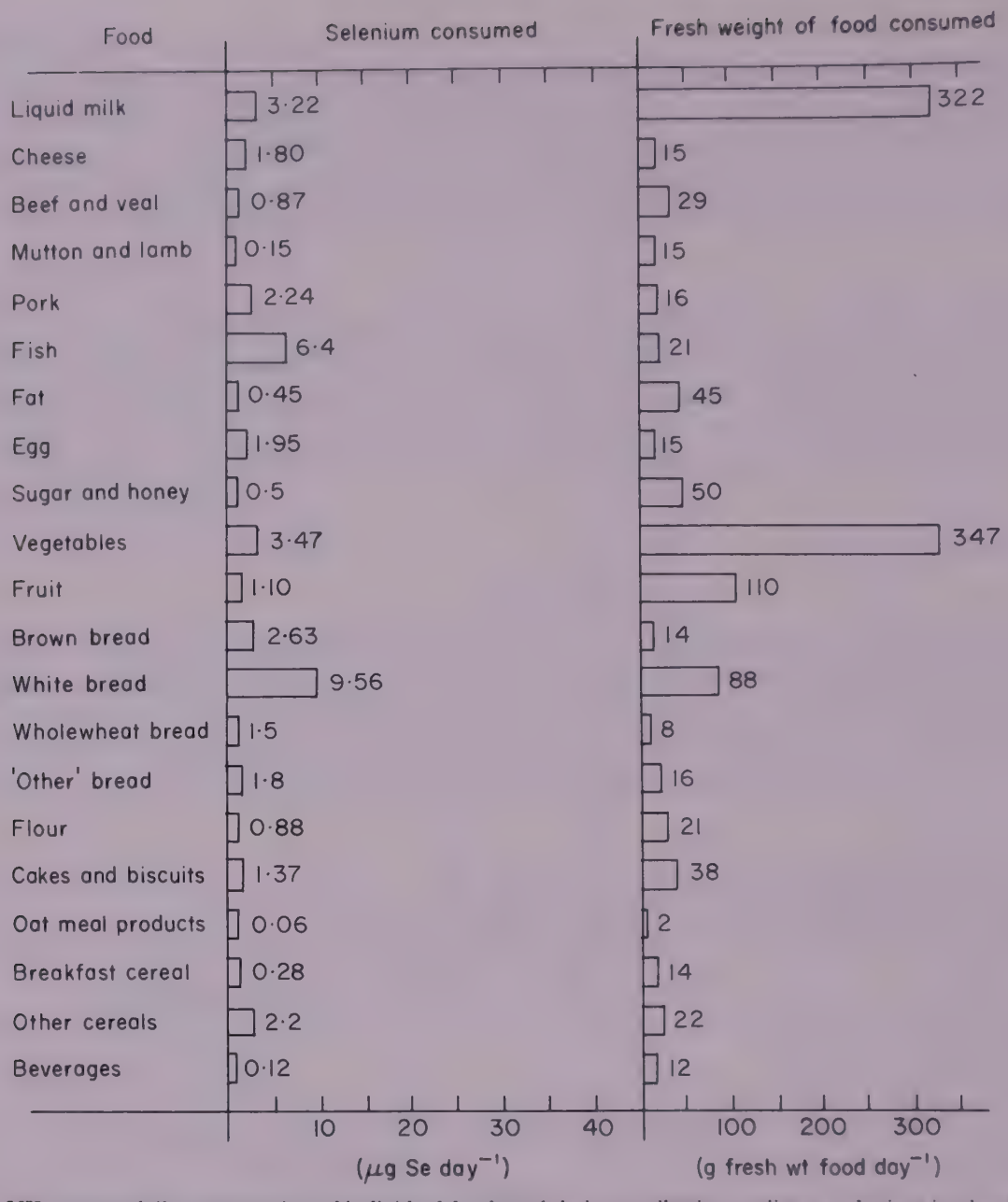

Figure 2. UK average daily consumption of individual foods and their contribution to dietary selenium intakes from MAFF National Food Survey 1982.

Table 3. Selenium content of flour and bread

	Flour	Selenium content ($\mu g\ g^{-1}$ fresh wt)	Bread	Selenium content ($\mu g\ g^{-1}$ fresh wt)
Barclay and MacPherson United Kingdom	Low protein white	0.042	Pan	0.036
	Medium protein white	0.143	Square	0.112
	Wholewheat	0.313	Wholewheat	0.188
Thorn *et al.*[6] United Kingdom	White	0.04		
	Bread-making	0.42		
	Wholemeal	0.53		
Morris and Levander[23] Maryland, USA	White	0.187–0.197	White	0.274–0.280
	Wholewheat	0.627–0.645	Wholewheat	0.654–0.676
Arthur[21] Canada	White	0.260–0.300	White	0.42–0.67
	Wholewheat	0.560–0.650	Wholewheat	0.65–0.71
Olson and Palmer[24] S. Dakota, USA	White	0.39	White	0.30
	Wholewheat	0.87	Wholewheat	0.41

Table 4. Regional total selenium intakes and the contribution to these from bread

Region	Total selenium μg Se day^{-1}	Selenium from bread μg Se day^{-1}
East Kilbride	46.3	18.5 (10.4)
Glasgow Garscadden	45.7	19.7 (9.5)
Scotland	43.7	16.9 (9.3)
Wales	43.1	16.8 (9.3)
England	44.7	15.9 (9.2)
North	48.7	17.5 (10.1)
Yorkshire and Humberside	46.5	16.1 (9.4)
North West	45.3	17.9 (9.7)
East Midlands	44.9	15.9 (9.1)
West Midlands	45.9	17.2 (8.6)
South West	42.9	13.3 (8.1)
South East Anglia	43.1	14.4 (8.9)
Greater London	43.2	14.1 (8.8)
Metropolitn areas including Clydeside	46.8	17.8 (9.6)

Figures in parentheses based on use of low protein flour for bread-making with an average Se content of 0.036 μg g^{-1}.

supported by Olson and Palmer[24] but not by Morris and Levander[23] or Arthur[21] who reported higher bread selenium levels than those in the corresponding flours.

Table 4 shows the regional variation of total selenium intakes and the contribution to these from bread. For white breads made from *medium* protein flours, bread selenium content and daily selenium intake from bread are calculated to be 0.112 μg g^{-1} and 13.3–19.7 μg day^{-1} respectively. Corresponding figures for white bread made from *low* protein flours are 0.036 μg g^{-1} and 8.1–10.4 μg day^{-1}. Total daily selenium intake may fall from 43 μg day^{-1} to 35 μg day^{-1} (i.e. by 16.3%) as a result of the change from medium to low protein flours. The MAFF annual nutrition figures show the UK average consumption of bread to be falling from 1.29 kg week^{-1} in 1960 to 0.880 kg week^{-1} in 1982 and 0.871 kg week^{-1} in 1983. This trend compounded with the move to UK and lower protein wheats will significantly decrease the selenium intake.

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